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Distribution and Shape Analysis of *Phorcus lineatus* and *Phorcus sauciatus* along the Portuguese Coast

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RESUMO

As costas rochosas estão entre os locais mais extremos, heterogéneos e dinâmicos da Natureza, sujeitos a condições extremas que influenciam a distribuição espacial e a colonização de inúmeras espécies. Nestas zonas ocorrem dois principais gradientes ambientais: um horizontal - presente ao longo da linha da costa - e um vertical - causado pela interface entre o ar e a água. Ao longo do gradiente horizontal existe um gradiente de exposição à acção das ondas, que é fortemente influenciado pela topografia da costa, nomeadamente pela presença de cabos, nos quais um dos lados está mais protegido do hidrodinamismo. Assim, cabos são locais apropriados para estudar a adaptação dos organismos às diferentes condições hidrodinâmicas, exactamente por proporcionarem habitats contrastantes no que toca ao hidrodinamismo.

A exposição à vaga tem um efeito profundo nas características ecológicas e biológicas de uma costa, afectando a distribuição dos organismos. Uma crescente exposição conduz a um crescente risco de desprendimento e danos físicos, limitando assim a distribuição de espécies mais frágeis e susceptíveis. Nos locais mais expostos, apenas os organimos capazes de se manterem agarrados nas rochas são encontrados.

As conchas constituem um registo ontogenético dos indivíduos. O estudo da forma das conchas em relação a factores ambientais permite assim perceber como determinado factor influencia a forma da concha. Em espécies próximas, uma variação na forma das conchas pode indicar uma variação dos seus hábitos de vida. Neste caso, estas diferenças na forma poderão indicar diferentes respostas para a mesma pressão selectiva, bem como diferenças em processos de crescimento e morfogénese, uma vez que é espectável que pressões ecológicas semelhantes resultem no desenvolvimento de características morfológicas semelhantes.

Dos grupos de organismos existentes na zona intertidal, os gastrópodes são dos mais abundantes e bem representados. Para além de facilmente identificáveis, apresentam diferenças nas conchas que são mensuráveis, por exemplo, forma ou tamanho, que podem ser relacionadas com um determinado factor ambiental.

Phorcus Risso 1826 é um género de troquídeo herbívoro (Gastropoda Prosobranchia) que habita as costas rochosas, desde o Mar Mediterrâneo até ao Atlântico Norte. Na costa portuguesa destacam-se *Phorcus lineatus* (da Costa, 1778) e *Phorcus sauciatus* (Koch, 1845).

P. lineatus é maioritariamente encontrado em zonas com poças e fendas, protegidas da acção directa da vaga, geralmente na zona supratidal. Esta espécie tem o limite norte de distribuição entre o País de Gales e Irlanda. A sua distribuição é contínua ao longo da costa de França, Espanha e Portugal, atingindo o seu limite sul em Marrocos. *P. sauciatus* é uma espécie subtropical que atinge a limite norte da sua distribuição na Peninsula Ibérica. Geralmente são encontrados em plataformas rochosas de inclinação suave, geralmente mais expostas ao hidrodinamismo.

Neste contexto, com a presente dissertação pretende-se realizar um estudo morfométrico da concha destas duas espécies na costa continental portuguesa, e desta forma aumentar o conhecimento neste tema, que é até à data algo restrito, especialmente em *P. sauciatus*. Foram abordadas as seguintes questões: (1) Como varia a abundância das duas espécies ao longo da costa?; (2) Qual a influência da latitude na variação do tamanho e forma da concha?; (3) Qual a influencia da exposição da costa no tamanho e forma da concha?; e (4) A forma da concha varia durante a ontogenia?

Para isso, foram recolhidos indivíduos de *Phorcus lineatus* e *Phorcus sauciatus* em 29 locais da costa Portuguesa, no lado norte e no lado sul de vários cabos, desde a praia da Papoa (39°22'12.51"N / 9°22'38.72"O) à praia da Luz (37° 5'5.96"N / 8°43'1.94"O), num gradiente geográfico norte-sul de aproximadamente 400km.

Cada um dos 2193 espécimes recolhidos nos 29 locais foi identificado, medida a altura máxima e a largura máxima da concha (medidas de abordagem tradicional), e feito o registo fotográfico em condições constantes (para posterior análise de abordagem geométrica).

Para o estudo geométrico geográfico foram escolhidos 50 espécimes de cada local, com uma largura entre 13 mm e 17 mm. Para o estudo geométrico ontogenético, foi escolhida uma população de cada uma das duas espécies, e os espécimes foram divididos em quatro classes de largura, cada uma com 50 espécimes: I- [1 mm - 5 mm], II- [6 mm - 10 mm], III- [11 mm - 15 mm], IV- [16 mm - 20 mm], referidas daqui em diante como classes de tamanho.

A morfometria geométrica é a caracterização quantitativa, análise e comparação das formas biológicas. Foi utilizado o método de Procrustes, que assenta na translação, escalamento e rotação da informação morfométrica, removendo toda a informação não relacionada com a forma do indivíduo. Este método demonstra as diferenças entre uma forma de referência e a forma alvo, evidenciando a dispersão de pontos (*landmarks*) marcados homologamente na forma alvo, ou seja, nos exemplares que se pretende analisar. Deste modo, foram marcados 14 pontos homólogos, na fotografia de cada exemplar, para posterior análise no software de análise morfométrica geométrica.

Utilizou-se uma análise de covariância (ANCOVA) nas medidas tradicionais, e uma análise de variância aninhada multivariada (MANOVA), nos valores RW (RW scores), outputs da análise geométrica.

A variação da forma das conchas foi então estudada em dois níveis: Geométrico e Ontogenético. No primeiro realizou-se uma abordagem em relação à latitude, abrangendo todas as praias ao longo da costa, e em relação à exposição à vaga. Neste último caso, de modo a perceber o efeito do hidrodinamismo a diferentes escalas espaciais, foi considerando o efeito da orientação da costa a norte ou a sul dos cabos, independentemente do cabo; o efeito da orientação da costa em cada cabo, e ainda um estudo numa escala mais restrita, comparando duas populações de um mesmo local.

Como esperado, houve uma predominância clara de *P. lineatus* no norte e de *P. sauciatus* no sul. Ingrina foi uma excepção - localizada a sul, nesta praia *P. lineatus* foi mais abundante. Neste caso, a latitude poderá não ser o principal factor a influenciar a distribuição destas espécies, mas sim a exposição e tipo de substrato.

Em relação ao estudo latitudinal, a morfologia da concha não apresentou um padrão claro quando consideradas todas as populações da costa. No entanto, considerando o Método Tradicional, em *P. lineatus* foi, ligeiramente, perceptível um gradiente geográfico - houve uma tendência para conchas mais largas e mais altas nas populações do norte, e indivíduos de menores dimensões no sul. Diferentemente, *P. sauciatus* apresentou uma largura mais uniforme, não revelando assim qualquer padrão evidente. A abordagem Geométrica não demonstrou qualquer padrão claro ao longo da costa, mas, no entanto, alguns locais apresentaram-se claramente distintos de outros. Tendo em conta que a extensão latitudinal da costa Portuguesa é reduzida, e que ao longo da costa existem inúmeros acidentes geomorfológicos que influenciam a exposição costeira e que tornam a costa Portuguesa muito heterogénea, é de facto difícil observar um um padrão de forma claro.

Considerando o efeito da exposição à vaga na morfologia da concha, através da abordagem Tradicional, e tendo em conta os efeitos integrados de orientação da costa, os indivíduos de ambas as espécies são ligeiramente mais largos e achatados no lado norte dos cabos, onde, devido à ondulação oceânica dominante noroeste (*swell*), a exposição à vaga é mais forte. No que diz respeito à variação em cada cabo e em duas populações de dois lados diferentes da mesma praia, a forma das duas espécies foi muito variável, e um padrão foi visível apenas em poucos casos. Relativamente à abordagem geométrica não foi de todo visível uma diferenciação clara de forma da concha.

A variabilidade observada na morfologia da concha de ambas as espécies poderá indicar que a exposição à vaga e a latitude não são suficientes para moldar estas espécies diferentemente.

Em relação ao estudo morfométrico da ontogenia, em ambas as espécies, à medida que os indivíduos crescem, a relação entre largura e altura torna-se mais dispersa. No que diz respeito à sua forma foram encontradas diferenças ao longo a ontogenia, com classes de tamanho a variar a sua forma à medida que os seus hábitos mudam.

São inúmeros os factores que influenciam a morfologia da concha, e sua interacção pode ter conduzido a uma falta de um padrão evidente, uma vez que não foram considerados no presente estudo.

Palavras chave: Morfologia, Exposição à vaga, Ontogenia, *Phorcus lineatus*, *Phorcus sauciatus.*

ABSTRACT

Exposure has a deep effect on the biological and ecological characteristics of a shore, affecting the distribution of organisms. The level of wave exposure is deeply influenced by shore topography, for example, the presence of capes shelters part of the coast from the wave action. Capes are suitable places for studying organisms' adaptation to the wave action since they provide contrasting situations of wave exposure.

Phorcus Risso 1826 are herbivorous trochids (Gastropoda Prosobranchia) present from the Mediterranean Sea through the North Atlantic. In the Portuguese coast the dominant species are *Phorcus lineatus* (da Costa, 1778) and *Phorcus sauciatus* (Koch, 1845). *Phorcus lineatus* is mainly found in areas with rock pools, cracks, crevices or under boulders of the upper half of the intertidal zone. This species reaches its northern limits in Wales and Ireland and its distribution is continuous from the French coastlines until Morocco. *Phorcus sauciatus* is a common subtropical grazer that reaches its northern boundary in the Iberian Peninsula and usually inhabits extensive and gently sloping rocky platforms.

In this study it was intended to uncover differences in the morphology of these two species from different locations along the Portuguese coast. Four questions were addressed: How does the abundance of these species vary along the Portuguese coast? ; What is the influence of latitude on the variation of shell size and shape? ; What is the influence of shore exposure on shell size and shape variation? ; Does shell shape vary during ontogeny?

To answer these questions, *P. lineatus* and *P. sauciatus* were collected from 29 sites, in the northern and the southern side of several capes, ranging a geographical north-south gradient of 400 km along the Portuguese west coast. The collected specimens were identified, measured (maximum height and maximum width, traditional morphometric approach) and photographed (geometric morphometric approach).

Firstly, shell morphology was analysed in a geographic perspective, evaluating the influence of latitude and exposure to wave action on the shell shape. Concerning wave exposure, three scales were considered: the integrated effects of shore orientation, the effects of orientation in each cape, and a short scale comparison of two sides of the same beach.

Secondly, considering one population of each species, shell morphology was analysed in an ontogenetic perspective, in which specimens were compared regarding four size classes.

As expected, there was a clear dominance of *P. lineatus* in the northern coast and of *P. sauciatus* in the southern coast. Ingrina was one exception - in this southern beach *P. lineatus* was more abundant. In this case, latitude may not be the major factor influencing these species distribution, but rather exposure and type of substrate.

Regarding the latitudinal study, shell morphology did not show a clear pattern when considering all populations from the Portuguese coast. Considering the traditional method, however, in *P. lineatus* a geographic gradient was apparent in some extent - there was a tendency for broader and higher shells in the northern populations, and smaller individuals in the south. Differently, *P. sauciatus* presented a more uniform width, revealing no evident pattern. The geometric approach did not show any clear pattern throughout the coast but there were some locations clearly distinct from the others. Since the latitudinal range of the Portuguese coast is not very wide, and throughout the coast there are several geomorphological accidents that influence the coastal exposure, making the Portuguese coast very heterogeneous, a clear shape pattern is difficult to observe.

Concerning the effect of wave exposure on shell morphology, through the traditional approach, and considering the integrated effects of shore orientation, specimens of both species are slightly broader and flatter in the north side of the capes where, due to the dominant northwest oceanic swell, the exposure is stronger. In the smaller scales studied, i.e. variation in each cape and in two populations from two different sides of the same beach, the shape of the two species was very variable, and a pattern was only visible in few cases. Regarding the geometric approach a clear differentiation of populations' shell shape was not found.

The variability found in the shell morphology of both species indicates that neither the exposure nor the latitude is enough to shape these species differently.

Regarding the ontogenetic morphometric study, in both species, as individuals grow, the relation between width and height becomes more disperse. As for their shape, differences throughout the ontogeny were found, with size classes varying their shape as their life habits change.

Several factors definitely influence shell morphology, and their interaction may lead to a lack of an evident pattern, since they were not all considered in the present study.

Key Words: Morphology, Wave Exposure, Ontogeny, *Phorcus lineatus*, *Phorcus sauciatus*.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

INTRODUCTION

Intertidal Rocky Shores

Rocky shores are among the most extreme, heterogeneous and dynamic environments in nature (Johannesson 2003; Raffaeli & Hawkins 1996) subject to extreme conditions that influence spatial distribution and colonization of many species (Ramirez et al. 2005). Dependent on their geology, rocky shores can be crossed with cracks, crevices, gullies and pools (Raffaelli & Hawkins 1996).

The rocky shores have two main environmental gradients: a horizontal one, along the coastline; and a vertical one. Although intimately associated with tides, the vertical gradient is not generated by them, but by the interface between air and water. Tides simply amplify this existing gradient by moving the water's limit up and down, thereby increasing the amount of living space for organisms (Raffaelli & Hawkins 1996). Therefore, along the vertical gradient there is a unidirectional stress, where the environmental conditions (such as emersion periods, wave action, temperature, salinity, and irradiation) vary from a completely aquatic environment to a completely terrestrial one, creating an extremely heterogeneous habitat, and consequently subjecting organisms to different levels of thermal and hydric stress (Ramirez et al. 2005).

Along the horizontal axis there is a gradient of exposure to the wave action, from sheltered bays to exposed headlands. This gradient exists both among microhabitats within shores and between different shores, affecting organisms mainly by physical disturbance in the water (Lindegarth & Gamfeldt 2005). The extent of wave action on the shore is determined by the aspect to the dominant winds coupled with the 'fetch', that is the distance over which the wind blows (Raffaeli & Hawkins 1986). Shores with a longer fetch have stronger wave action because the wind has a greater distance to create the height of the waves (Raffaeli & Hawkins 1986).

The interaction between these two gradients deeply influences organisms' distribution in rocky shores. Species are distributed depending on their physical and physiological tolerance for all these environmental factors, developing various adaptations to withstand the different hydrodynamic conditions (Raffaeli & Hawkins 1996; Lindegarth & Gamfeldt 2005). This distribution is called 'zonation', and it can be recognized along rocky shores (Stephenson & Stephenson 1949; Raffaeli & Hawkins 1996). Zonation is therefore influenced by many factors, including wave exposure, temperature, salinity, substrate orientation and composition, and tidal dynamics (Stephenson & Stephenson, 1949; 1972). Due to this complexity, variation in one factor often masks the effects of another (Harley & Helmuth 2003).

Coastal setup of the Portuguese Coastline

Geographic location can be considered an important and all-embracing environmental gradient (Raffaelli & Hawkins 1996). Located between three important biogeographic areas - the cold temperate Atlantic, the warm temperate Atlantic and the Mediterranean - the Portuguese coast has a very interesting biogeographical context, constituting an important transition zone, an interface between distinct biogeographic provinces, where marine species with boreal, temperate and subtropical affinities occur in sympatry (Marques et al. 2006). The Nazaré Canyon, in Peniche, is one of the largest submarine canyons of Europe, intersects the Portuguese continental margin between 39°20’N and 40°N (Stigter et al. 2007; Tyler et al. 2009), and it is considered a biogeographic limit to several marine species thus, including the transition zone of the biogeographic province, by the Nazaré canyon, to the south coast.

The west Portuguese coast extends along the 9°W meridian between 37°N and 42°N, and the south coast (Algarve) is oriented along 37°N, between 7°20'W and 9°W (Fiúza et al. 1982). On the western Portuguese coast, sea surface temperature (SST) reveals marked seasonality, varying between 13 and 15^{o}C during winter, and reaching 20^{o}C or more during summer. On the south coast, temperatures are in general slightly higher (approximately 1 - 1.5^{o}C) (Boaventura et al. 2002; Fiúza et al. 1982), a difference even more marked during summer.

Oceanographic current systems are known to represent a major influence on coastal ecosystems as they are largely responsible by the temperature and nutrient status of the adjacent waters (Raffaeli & Hawkins 1996), which influences the distribution of organisms throughout the shore. Therefore, besides the northerly wind-induced upwelling occurring from July to September (Boaventura et al. 2002; Fiúza et al. 1982; Lemos & Pires 2004), which has a marked influence on shore communities (Raffaeli & Hawkins 1996), this temperature variation may be explained by the fact that the west Portuguese coast is under the direct influence of the eastern limb of the North Atlantic Subtropical Gyre - the Portugal Current- which brings colder water from higher latitudes. On the contrary, the coast of Algarve is sheltered from this circulation and upwelling is intermittent and short-lived (Barton 2001), so its effects are felt with much less intensity (Fiúza et al. 1982).

Another important factor in oceanographic feature of the Portuguese coast that may contribute to these different temperatures between the west and south coast of Portugal is the swell. Due the predominant northwest oceanic swell, the mean wave conditions are similar throughout the west coast, reaching values of over 5 m during winter. The south coast it's not exposed to that swell, and consequently the wave conditions are less severe, reaching only 3 m on the same season (Boaventura et al. 2002).

Wave Exposure

Exposure means how much wave action a shore experiences (Raffaelli & Hawkins 1996). In marine intertidal habitats, the importance of this variable as a structuring agent is widely agreed upon (Lindegarth & Gamfeldt 2005). It has a deep effect on the biological and ecological characteristics of a shore (Lindegarth & Gamfeldt 2005; Raffaelli & Hawkins 1996), influencing species' biology and ecology (McQuaid & Lindsay 2007; D'Amours & Scheibling 2007). Wave action affects organisms directly through mechanisms such as physical disturbance (Vadas et al. 1990), fluxes of propagules and nutrients, and sedimentation (Lindegarth & Gamfeldt 2005). Indirectly, it can cause mortality of juvenile and adult assemblages through dislodgement of algae, rocks and debris (Vadas et al. 1990). Indirect effects are often more subtle and can be easily mistaken with the impacts of other variables such as tidal height, biotic interactions, temperature, etc. (McQuaid & Lindsay 2007; Harley & Helmuth 2003).

Exposure to wave action affects the distribution of organisms (Lindegarth & Gamfeldt 2005). Exposed conditions are favourable for suspension feeders and sessile predators, since the water movement allows the flow of food and preys to these organisms (Raffaelli & Hawkins 1996). However, increasing exposure involves an increased risk of dislodgement and physical damage, limiting the range of susceptible and physically fragile species. In the most exposed situations, only those able to maintain a firm hold on rocky surfaces are found (Trussell 1997). For some species the attachment to the shore, or even foraging, is difficult in wave exposed areas, and sheltered conditions are more favourable (Raffaelli & Hawkins 1996).

The level of wave exposure is deeply affected by shore topography, for example, capes and headlands shelter part of the coast from the wave action by protecting one of its sides. In each side of a cape the hydrodynamic conditions are distinct, for example throughout the Portuguese west coast, the capes' northern side is under the direct influence of the northwest oceanic swell, and the southern side is more sheltered from this exposure. Phenotypic plasticity, which is the capacity of an organism to produce different phenotypes in response to environmental cues, can be an important adaptive strategy in variable environments (Trussell & Smith 2000). Thus capes are suitable places for studying organisms' adaptation to the wave action, because different conditions can lead to different adaptations, and consequently to different phenotypes.

Morphological Variation

Shells represent ontogenetic records of their owners. In closely related species, shell shape differences may indicate a variation of their life habits (Stone, 1998). In other words, differences in shape may indicate different responses to the same selective pressures, as well as differences in processes of growth and morphogenesis (Zelditch et al. 2004), since similar ecological pressures are expected to result in similar development of morphological characters (Madeira et al. 2012).

The shell morphology of different species of high-intertidal gastropods from several different shores has been shown to vary according to the degree of exposure to wave action (Rolán et al. 2004), revealing the existence of morphological gradients (Vermeij 1973). These morphological variations are also believed to be responses to selective pressures of other factors such as desiccation and predation (Crothers 2001; Fletcher 1995). At species level, shell shape variation may also occur in a microgeographical scale (between different shore levels at the same local) and in a macrogeographical scale (between distant regions) (Avaca et al. 2013).

Other source of variability in shell morphology may be related to the presence of allometric patterns (Avaca et al. 2013). Allometry is the variation of features associated with variation of the overall size of organisms (Huxley & Teissier1936). The study of allometry is interesting not only because of its implications for form-function relationship but also because of the insight into growth and development (Zelditch et al. 2004).

Understanding the origin of morphological variation, and searching for an explanation for it, is a major research focus, including fields as diverse as functional morphology, macroevolution, sexual selection, and evolutionary developmental biology (Rosenberg 2002). Explanations for biological form are multiple and complex (Roth & Mercer 2000). Shape analysis is one approach to understand those diverse causes of variation and morphological transformation (Zelditch et al. 2004), playing an important role in biological studies (Silva et al. 2009; Zelditch et al. 2004). Roth & Mercer (2000) claim that morphometrics has provided a first step on a path of inference that leads from morphology to explanations (such as natural selection and pleiotropy) drawn from other disciplines.

Morphometric Analysis

Geometric morphometrics is the quantitative characterization, analysis, and comparison of biological shape (Roth & Mercer 2000). These techniques have many advantages including the possibility to discriminate shape and size components of morphology, the graphic display of results and their easy interpretation, the preservation of geometric relationships throughout the analysis and a notable statistical power (Bookstein et al. 1985; Rohlf & Slice 1990; Avaca et al. 2013). Furthermore, geometric morphometrics allows us to easily focus on shape variation, avoiding the problems of more traditional schemes that measure size rather than shape (Silva et al. 2009).

Geometric methods can be subdivided into two principal categories: deformation methods and superimposition methods (Roth & Mercer 2000). In the latter group is included the Procrustes method, which is one of the most widespread and best understood in its mathematical and statistical properties (Mitteroecker & Gunz 2009), and the one that more directly preserves geometry and the spatial relationships among landmarks and contours of a form (Roth & Mercer 2000).

The Procrustes method relies on translation, scaling, and rotation, removing all information unrelated to shape (Zelditch et al. 2004) and it is based in the recognizable discrete anatomical loci, named landmarks, which can be found repeatedly and reliably in all specimens (Zelditch et al. 2004; Avaca et al. 2013). Landmarks not only have their own locations but also have the ‘’same’’ locations in every other form of the sample and in the average of all the forms (Vermeij 1973). Procrustes method preserves the geometry of the landmark configurations throughout the analysis and thus allows to represent statistical results as actual shapes (Mitteroecker & Gunz 2009).

The relative warps can be used as classical morphometric variables in statistical univariate and multivariable analysis and tests (Zelditch et al. 2004). Furthermore, there is a possibility to represent the geometric meaning of the relative warps, using the thin-plate spline representation method (Carvajal-Rodríguez & Guerra-Varela 2006).

Studied Species

Intertidal gastropods have proved to be especially adequate in population differentiation studies mostly because they inhabit heterogeneous environments and exhibit conspicuous variation in morphology, life-history and behaviour (Fletcher 1984; Johannesson et al. 1993; Rolán et al. 2004; Trussell & Smith 2000).

Phorcus Risso 1826 is a genus of herbivorous trochids (Gastropoda Prosobranchia) that inhabits rocky shores. It is easy to find and to identify; it shows differences in measurable features (such as abundance, size and shape) that can be related to a measurable environmental variable (such as wave action and latitude) (Crothers 2001). Because of all these factors, this genus is particularly suitable for morphological variation studies, within and between populations.

There are 9 living species in the *Phorcus* genus (Donald et al. 2012). In this work we studied the two most abundant on the Portuguese coast, *Phorcus lineatus* (da Costa 1778) and *Phorcus sauciatus* (Koch 1841).

P. lineatus is mainly found in areas with rock pools, cracks, and crevices, and sometimes under boulders (Crothers 2001; Pedro 2004), being found grazing in the upper half of the intertidal zone (Desai 1966). This species extends much further north than any other species of *Phorcus* (Crothers 2001), reaching its northern distribution limits in Wales and Ireland (Kendall 1987; Pedro 2004; Donald et al. 2012). Its distribution is continuous along the Portuguese, Spanish and French coastlines, and its southern limits are reached in Morocco (Mieszkowska 2005). *Phorcus sauciatus* is a common subtropical grazer that reaches its northern boundary in the Iberian Peninsula (Rubal et al. 2014). Usually this species inhabits extensive, and gently sloping, rocky platforms (Ramírez et al. 2009).

Similarly to several gastropod species (Serbina 2010), in *P. lineatus* and *P. sauciatus* size and age are positively related, thus allowing to investigate population structure, growth rates, mortality rates and longevity (Crothers 1998).

P. lineatus breeding stages occur from June-July to September, and in some individuals it happens until November (Bode et al. 1986). Spawning occurs between May and August (Graham 1988). The larva hatches and settles after 4-5 days, low on the beach, migrating later to a higher level. The new settlers reach around 5-6 mm shell length by their first autumn (Crothers 1994; 2001), while their predecessors (i.e., snails one year older) can grow from 6 mm to 14 mm (Crothers 1994). After a winter interruption caused by the low temperatures

(Crothers 2001; Mannino et al. 2008), which slow metabolism down, growth continues rapidly through the year, before slowing again for the second winter (Crothers 2001). During their first year (apart from winter interruptions) growth is fast (Williams 1965), decreasing in their second and, thereafter, individuals grow about a millimetre a year (Crothers 1998; 2001). This abrupt stop in growth means that the animal has reached sexual maturity (Graham 1988; Crothers 1994; 2001). Molluscs in general can only grow if they conduct the majority of their energy to shell production. Reaching adult phase, most of their energy is used in reproduction (Crothers 1994), leaving little left to growth. *P. lineatus* individuals have been known to reach 34 mm in shell height and 15 years of age (Crothers 1998).

P. lineatus is a well-studied species with various studies about it. Contrary, very few studies addressed *P. sauciatus* about population structure, size or growth. The only information is that its size ranges from 5 to 26 mm (Ramírez et al. 2009). Yet, since they are closely related species, the data from *P. lineatus* can be extrapolated to *P. sauciatus.*

Intertidal gastropods from exposed areas have thin and smooth shells with wide apertures (Rolán et al. 2004), due to the large foot required to cope with the higher risk of wave displacement (Trussell 1997). In sheltered conditions they have thick shells with a narrow aperture, to resist desiccation (Raffaelli & Hawkins 1996, Tuya et al. 2005). This pattern can be seen on these two species.

P. lineatus is used as an indicator of sheltered shores. In Ballantine's Exposure Scale (Ballantine 1961), where 1 corresponds to extremely exposed and 8 to extremely sheltered, this species is considered to be common on shores of exposure 6 and 7. On the contrary, exposure is not a major constraint for *P. sauciatus.* Apparently being more tolerant to wave action, it can be found lower on the shore than *P. lineatus* (Crothers, 2001; Rubal et al., 2014), but it is also found in sheltered shores (Rubal et al. 2014). *P.* sauciatus' larger foot, and consequently larger aperture, implies that this species is less tolerant to desiccation, since it has a larger surface in contact with air. Temperature also seems to play a major role shaping the range distribution of *P. sauciatus*. Low SST due to upwelling events seems to be responsible for the absence of this species in North Portugal and South Galicia (Rubal et al. 2014).

P. lineatus and *P. sauciatus* have approximately the same size. The shape of their shell is very similar, and the colour pattern on the outside can sometimes be misleading. A typical specimen of *P. lineatus* has a well-marked tooth on the columela and usually there is an umbilical scar. In *P. sauciatus* the tooth and umbilical scar are indistinct, and often there is a conspicuous dark purple mark in the umbilical area, and usually its shell is slightly flattened.

However there is always variation and atypical individuals, so the safest way to distinguish both species is looking to the inner colour of the aperture: in *P. lineatus* is always white, and in *P. sauciatus* there is always a coloured layer, that enters the whorl (Fig. 1**)**.

Fig. 1. A: two typical *Phorcus lineatus* individuals; B: typical *Phorcus sauciatus* specimen. Note the coloured layer of the shell is visible inside the aperture.

OBJECTIVES

This study examined the pattern of variation in size and shell shape among populations of *P. lineatus* and *P. sauciatus* throughout the Portuguese coast. The four following questions were addressed:

(1) How does the abundance of these two species vary along the Portuguese coast?

(2) What is the influence of latitude on the variation of shell size and shape?

(3) What is the influence of shore exposure on shell size and shape variation?

(4) Does shell shape vary during ontogeny?

It is intended to uncover differences in the morphology of these two species from different locations along the Portuguese coast. Within this context, the aim of the present dissertation is to perform a morphological study of these species, and therefore expand the current knowledge on the subject, which is quite spatially limited.

MATERIALS AND METHODS

1. Sampling design and Processing

Samples of *P. lineatus* and *P. sauciatus* were collected from 29 sites, from Papoa (39°22'12.51"N / 9°22'38.72"W), to Luz (37° 5'5.96"N / 8°43'1.94"W), ranging a geographical north-south gradient of approximately 400 km (Fig. 2) along the Portuguese west coast.

To analyse the influence of exposure on shell shape variation, sampling was performed in the northern and the southern side of several capes along the coast. Sampling was conducted between July 2013 and February 2014.

Individuals were captured in the mid-to-upper intertidal zone of the rocky shores, in the open substrate, small crevices and pools. To speed up the sampling process, as the available time window to work depended on the available low tide, specimens were randomly collected considering only their genus. Therefore, whenever possible, between 150 and 200 individuals of *Phorcus* spp. were captured, as a mean to ensure the collection of at least 50 individuals of each species (see_Table I). It is important to refer that due to the different distribution along the coast, at some sites, only one of the species, or very few specimens of the less abundant, were collected.

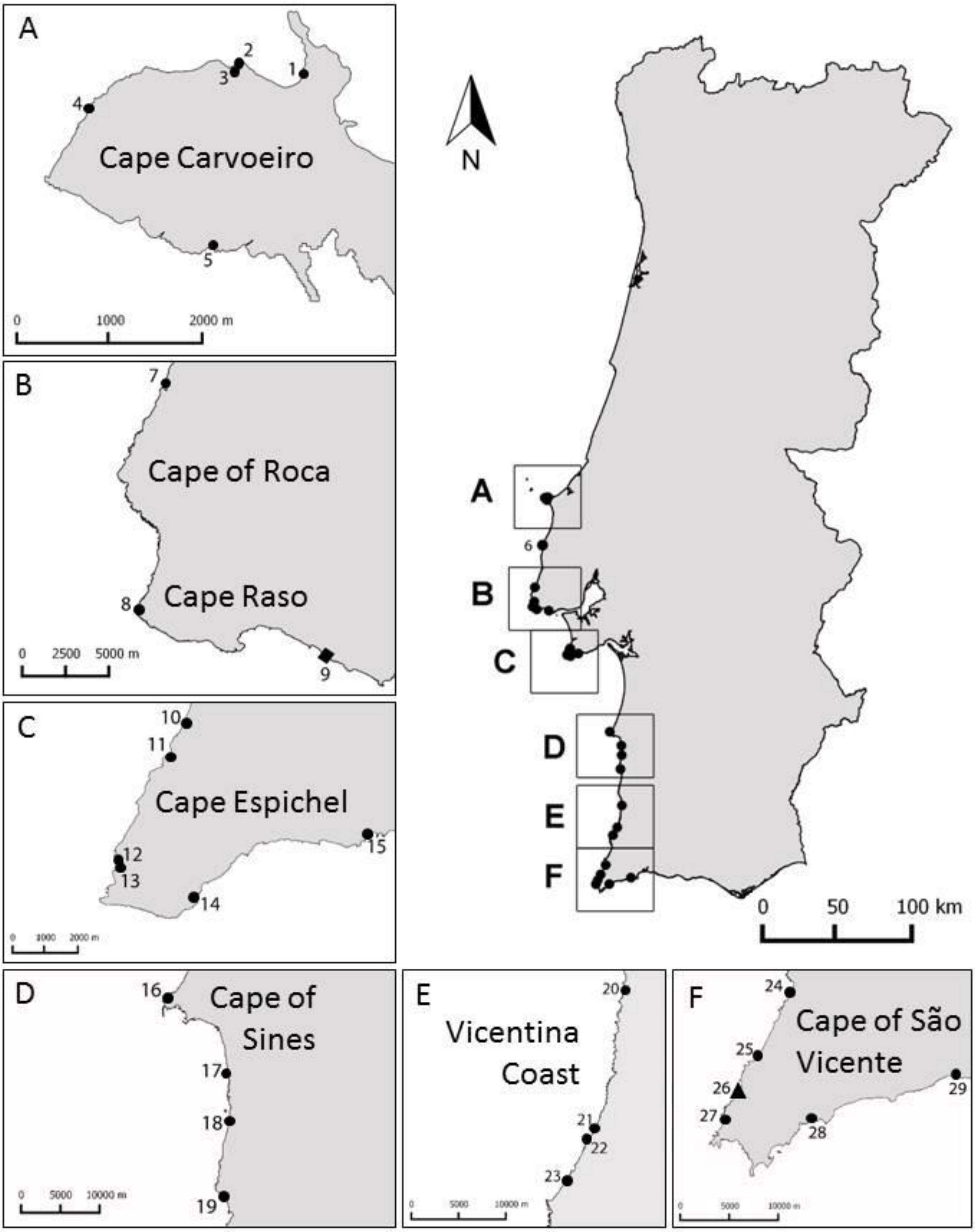


Fig. 2. Sampling locations for *P. lineatus* and *P. sauciatus* along the Portuguese coast: 1 - Papoa; 2- Portinho da Areia Norte East; 3 - Portinho da Areia Norte West; 4 - Cruz dos Remédios; 5 - Portinho da Areia Sul; 6 - Mexilhoeira; 7 - Maçãs; 8 - Cabo Raso; 9 - Avencas; 10 - Bicas; 11 - Foz; 12 - Lagosteiros North; 13 - Lagosteiros South; 14 - Porto da Baleeira; 15 - Ribeira do Cavalo; 16 - Pedras Amarelas; 17 - Burrinho; 18 - Queimado; 19 - Porto do Canal; 20 - Alteirinhos; 21 - Vale dos Homens North; 22 - Vale dos Homens South; 23 - Monte Clérigo; 24 - Amado; 25 - Cordoama; 26 - Ponta Ruiva; 27 - Telheiro; 28 - Ingrina; 29 - Luz. ● Samples used in the geographic morphometric study; samples also used in ontogenetic morphometric study: ♦ *P. lineatus* and ▲ *P. sauciatus*.

Table I. Number of specimens used in the geographic morphometric study from each sampling site.

Local	Species	
	P. lineatus	*P. sauciatus*
Papoa	50	0
Portinho da Areia Norte East	50	1
Portinho da Areia Norte West	41	0
Cruz dos Remédios	9	0
Portinho da Areia Sul	1	0
Mexilhoeira	50	0
Maçãs	50	1
Cabo Raso	50	41
Avencas	50	13
Bicas	3	50
Foz	3	50
Lagosteiros North	24	0
Lagosteiros South	13	50
Porto da Baleeira	30	50
Ribeira do Cavalo	50	0
Pedras Amarelas	50	50
Burrinho	50	50
Queimado	0	50
Porto do Canal	50	0
Alteirinhos	50	50
Vale dos Homens North	12	50
Vale dos Homens South	50	50
Monte Clérigo	50	50
Amado	50	50
Cordoama	19	50
Ponta Ruiva	27	50
Telheiro	1	50
Ingrina	50	24
Luz	50	50
Total	983	880

Table II. Number of specimens of each species in each size class used in the ontogenetic morphometric study.

Class	Species	
	P. lineatus	*P. sauciatus*
I	50	0
II	50	30
III	50	50
IV	50	50
Total	200	130

To prevent degradation, the captured specimens were frozen as soon as possible. Thereafter they were separated by species, counted, and then preserved in absolute ethanol. Given that the sampling was done randomly, only in the laboratory became known the exact number of individuals of each species.

Only at this point the population for the morphometric ontogenetic study of each species was chosen. The selected populations were the ones with greater size range, and with a large number of individuals, that would allow a division of the sample into four width classes (I- [1 mm - 5 mm], II- [6 mm - 10 mm], III- [11 mm - 15 mm], IV- [16 mm - 20 mm], hereafter referred as size classes), each one of these with 50 specimens (whenever possible) (see Table II). For *P. lineatus* the chosen population was from Avencas, and for *P. sauciatus* from Ponta Ruiva.

For the geographic morphometric study, the 50 specimens selected were, whenever possible, approximately of the same size (between 13 mm and 17 mm width).

Each of the 2193 specimens' shell from the 29 sites was measured (maximum height and maximum width, Fig. 3) and identified. Then they were photographed in adequate resolution digital images using a Nikon D70 digital camera with a 55 mm micro lens, maintaining consistent capture conditions. The specimens were photographed in apertural view, and were always placed in a consistent orientation, that is, with the shell axis parallel to the horizontal plane.

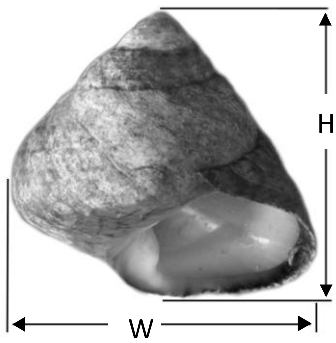


Fig. 3. Measures. W = Maximum Shell Width and H = Maximum Shell Height

2. Morphometric Analysis

The morphology of the shell was characterized in two dimensions, using geometric morphometric methods. These methods provide detailed information about the variation of the shape while preserving a visual representation of them throughout the analysis (Mitteroecker & Gunz 2009).

Firstly, the photographs of the specimens taken with the Nikon camera (*jpg* files) were converted into *tps* files using the tpsUtil 1.46 software (Rohlf 2010a). Then, 14 homologous points were chosen (Fig. 4) and digitalized in each image using the software tpsDig 2.16 (Rohlf 2010b). This procedure was performed in each species.

These points, "landmarks", are coordinates of points upon which geometric morphometric methods are based (Bookstein 1991), and were selected not only because they can be recognized in both species, but also for their relative ease in identification across samples and their capacity to describe the general shell shape.

Gastropods are known to have allometric growth (Fletcher, 1995; Vinarski, 2014; de Wolf et al., 1997), so it was necessary to evaluate the effect of size in shape variation. This was achieved using the tpsRegr 1.37 software (Rohlf 2009), by regressing each shape variable (named Relative Warps; RW) against a measure of body size and estimating the residual shape variation, which was then used for further morphometric analyses (Claverie et al. 2011).

Landmarks of each specimen were optimally aligned using Generalized Procrustes Analysis (Rohlf & Slice 1990), in which the configurations of the specimens are superimposed by translation, scaling and rotation, using the minimal bending energy method (Bookstein 1997; Mitteroecker & Gunz 2009; Rohlf & Slice 1990, Urra et al. 2007).

Centroid size, which is used as scaling factor during the superimposition process, was subsequently used as a measure of size for each individual. The term ‘shape’ used in this study is therefore defined as the geometric information that remains when location, scale and rotational effects are removed from an object (Avaca et al. 2013; Claverie et al. 2011; Madeira et al. 2012).

From the aligned specimens, shape variables were generated by performing a Relative Warp (RW) analysis (Bookstein 1991), which is the analogue of a principal components analysis (Zelditch et al. 2004).

RW analysis (Bookstein, 1991) describes shape variation as the deformation of landmarks from a reference configuration into those of each member in a group of specimens of interest (Stone 1998). This analysis reduces the dimensionality of multivariate data by transforming a set of many correlated variables into a small number of significant uncorrelated variables (Claverie et al. 2011), the relative warps. These new groups of shape variables were then used for statistical comparisons of shape variation within and between size groups and between populations. The present study also used the thin-plate spline approach, using the tpsRelw 1.49 software (Rohlf 2010c), which allowed the visualization of shape change as deformation grids.

This morphometric analysis was performed for the ontogenetic study and geographic study separately for each *Phorcus* species.

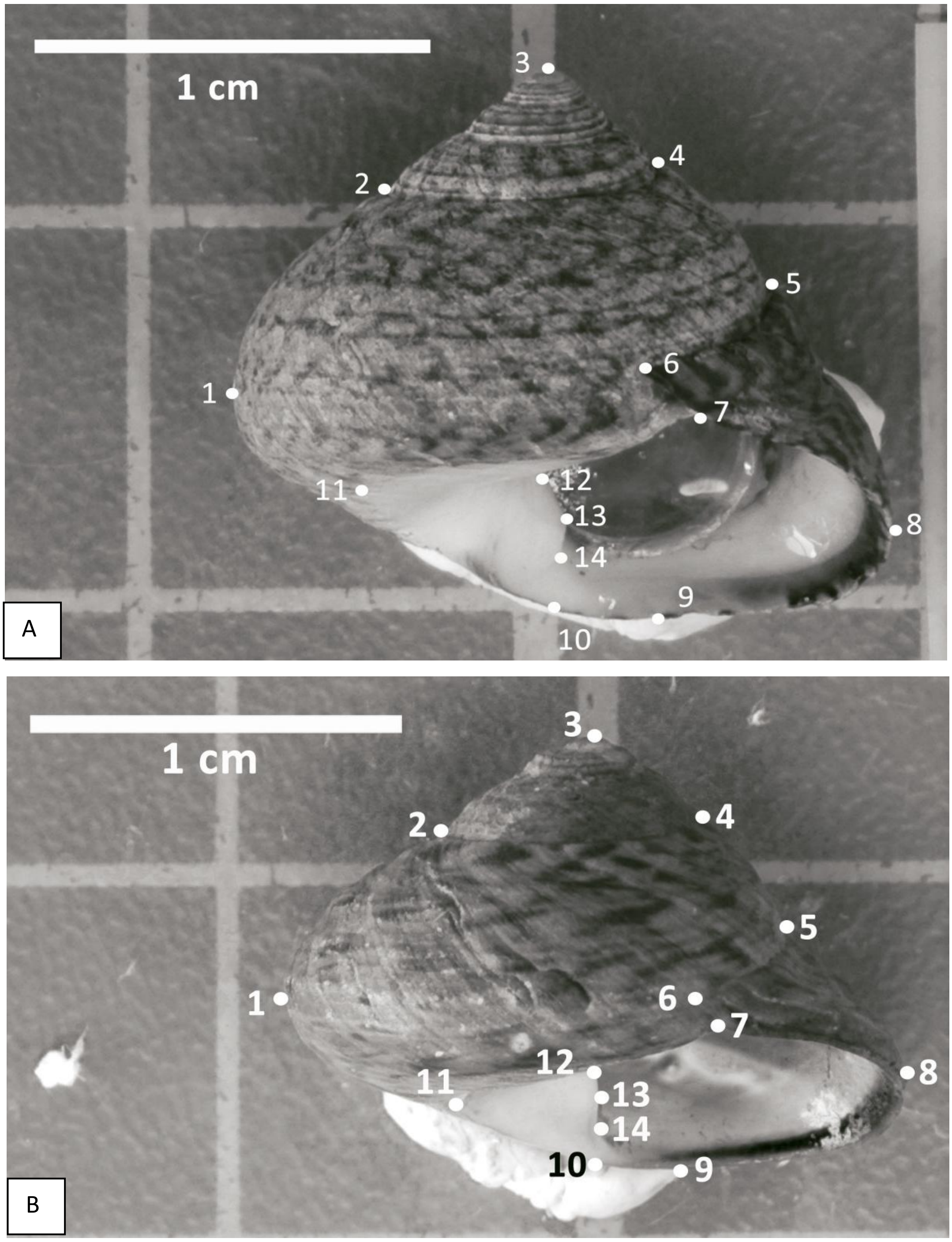


Fig. 4. Position of the 14 landmarks on the shell: LM1 - maximum curvature of the last whorl; LM2, LM4 and LM5 - sutures of the last and second whorls; LM3 - apex; LM6 - beginning of the shell's lip; LM7 - visual intersection of the lip with the basis of the first whorl; LM8 - most external point in the outer lip; LM9 - lowest point of the outer lip; LM10 - vertical projection of the apex; LM11 - edge of the nacreous surface; LM12, LM13 and LM14 - maximum indentation points above and below the columela's tooth. (**A -** *Phorcus lineatus*; **B** - *Phorcus sauciatus*).

3. Statistical Analysis

To investigate shell shape width/height variation significance, STATISTICA, version 10.0 (StatSoft Inc.) was used.

A covariance analysis (ANCOVA) was performed on the traditional measures, with shell width as the dependent variable, shell height as the continuous predictor and exposure/local as the categorical predictor.

A nested multivariate analysis of variance (MANOVA) was performed on the RW scores. In this way, it was determined the degree and significance of variation between capes, populations, and size classes. To test which population or size class differed within comparison, post-hoc Tukey's honestly significant difference tests were performed.

3.1. Geographic Morphometric Study

Shape Variation along the Coast

RW variation was analysed between all populations of each species, except populations with less than 10 individuals. For *P. lineatus* were disregarded the populations from Cruz dos Remédios (N=9), Portinho da Areia Sul (N=1), Bicas (N=3), Foz (N=3) and Telheiro (N=1). In *P. sauciatus* only two populations were disregarded: Portinho da Areia Norte East and Maçãs, both of them with only 1 individual. A MANOVA was performed on the RW scores, followed by the post-hoc Tukey's honestly significant difference test.

Using the same populations, an analysis of covariance was also performed on the height and width measurements.

Wave Exposure

◆Integrated effects of shore orientation

In this analysis it was intended to know if the wave exposure, accessed by the position on the North or South side of a cape influences the shell morphology of *P. lineatus* and *P. sauciatus*. In this way, throughout the coast, all locations in the north side versus all locations in the south side of the capes were analysed comparatively, regardless of the cape, using an ANCOVA on the width/height measures, and a MANOVA, on the RWs.

This analysis was performed using populations with more than ten individuals. Note that ideally the chosen populations would have 50 specimens. Whenever this wasn't possible, it was selected the population from the closest location, and with the specimen number close to 50.

◆ Effects of orientation in each Cape

The position in a cape and, consequently, the degree of exposure to the wave action may have an influence in the shell shape and width/height of *P. lineatus* and *P. sauciatus*. To infer this, RW scores were analysed, comparing locations on the north and south side of each cape (see Table III for *P. lineatus* and Table IV for *P. sauciatus*).

Table III. Selected *P. lineatus* populations

Phorcus lineatus

Cape	Local	N	Position North (N) / South (S)
Raso/Roca	Maçãs	50	N
	Cabo Raso	50	N
	Avencas	50	S
Espichel	Lagosteiros North	24	N
	Lagosteiros South	13	N
	Porto da Baleeira	30	S
	Ribeira do Cavalo	50	S
Sines	Pedras Amarelas	50	N
	Burrinho	50	S
São Vicente	Cordoama	19	N
	Ponta Ruiva	27	N
	Ingrina	50	S
	Luz	50	S

Table IV. Selected *P. sauciatus* populations

Phorcus sauciatus

space

Cape	Local	N	Position North (N)/ South (S)
Roca/Raso	Cabo Raso	41	N
	Avencas	13	S
Espichel	Bicas	50	N
	Foz	50	N
	Lagosteiros South	50	N
	Porto da Baleeira	50	S
Sines	Pedras Amarelas	50	N
	Burrinho	50	S
	Queimado	50	S
São Vicente	Cordoama	50	N
	Ponta Ruiva	50	N
	Telheiro	50	N
	Ingrina	24	S
	Luz	50	S

Beside the North-South location, it was also taken in account the number of specimens and the presence of both species on the capes (whenever the latter was not possible, it was selected the population from the closest location). The Cape Carvoeiro was disregarded in this analysis not only because *P. sauciatus* was absent, but also because all three *P. lineatus* populations were from the north side of the cape.

◆ Short scale - two sides in the same beach location

In this analysis it was intended to know if the position in two different sides of the same location influences the shell shape and width/height of *P. lineatus* and *P. sauciatus*. The locations were selected taking into account that they, if possible, should include specimens collected in two sides which exhibit different exposure degree, and that the specimens' number should be 50 each. For *P. lineatus* the selected populations were Portinho da Areia Norte East and Portinho da Areia Norte West, Lagosteiros North and Lagosteiros South, and Vale dos Homens North and Vale dos Homens South. Regarding *P. sauciatus* only one location was selected, Vale dos Homens North and Vale dos Homens South.

3.2. Ontogenetic Morphometric Study

To analyse shape variation and width/height variation through the ontogeny of *P. lineatus* and *P. sauciatus,* an ANCOVA and a MANOVA were performed on the four width classes of both species.

RESULTS

1. Abundance

Regarding the entire Portuguese coast, *P. sauciatus* has a relative abundance of 67.29%, and *P. lineatus* only 32.71%. As expected, due to the species' distribution (Crothers, 2001; Kendall, 1987; Pedro, 2004; Donald et al., 2012; Mieszkowska, 2005; Rubal et al., 2014), there is a clear dominance of *P. lineatus* in the northern coast and a dominance of *P. sauciatus* in the southern coast (Fig. 5). However, in Ingrina, and contrary to what was expected, *P. lineatus* was much more abundant than the southern species (*P. lineatus* with an relative abundance of 93.53% and *P. sauciatus* with 6.47%).

2. Morphometry - Traditional Approach

Accounting all sampling sites, and excluding the specimens used in the morphometric ontogenetic study, *P. lineatus'* mean shell height was 14.763 mm (± 1.638 mm, Standard Deviation - SD), and mean shell width was 16.189 mm (± 1.392 mm). As for *P. sauciatus*, the mean shell height was 12.283 mm (± 1.166 mm), and the mean shell width was 15.546 mm (± 1.041 mm) (see Table V).

Both species present broad shells, with mean width larger than mean height (Table V). However in *P. sauciatus* this difference was much more clear than in *P. lineatus*: the mean difference between shell width and shell height was 1.437 mm ± 0.246 mm in the latter, and 3.263 mm ± 0.125 mm in *P. sauciatus*, which translates to a ratio of 1 mm height : 1.097 mm width and 1 mm height : 1.266 mm width, respectively. In *P. lineatus*, the maximum shell height and width was found in one specimen from Cruz dos Remédios, with 34.49 mm high and 30.59 mm broad. The smallest one was sampled in Avencas, with 1.93 mm high and 2.27 mm broad. Regarding *P. sauciatus*, the smallest specimen was found in Ponta Ruiva, with 5.05 mm high and 7.75 mm broad. The largest shell width (22.26 mm broad and 18.29 mm high) was found in Pedras Amarelas, and the largest height (19.92 mm high and 21.98 mm broad) was from Avencas.

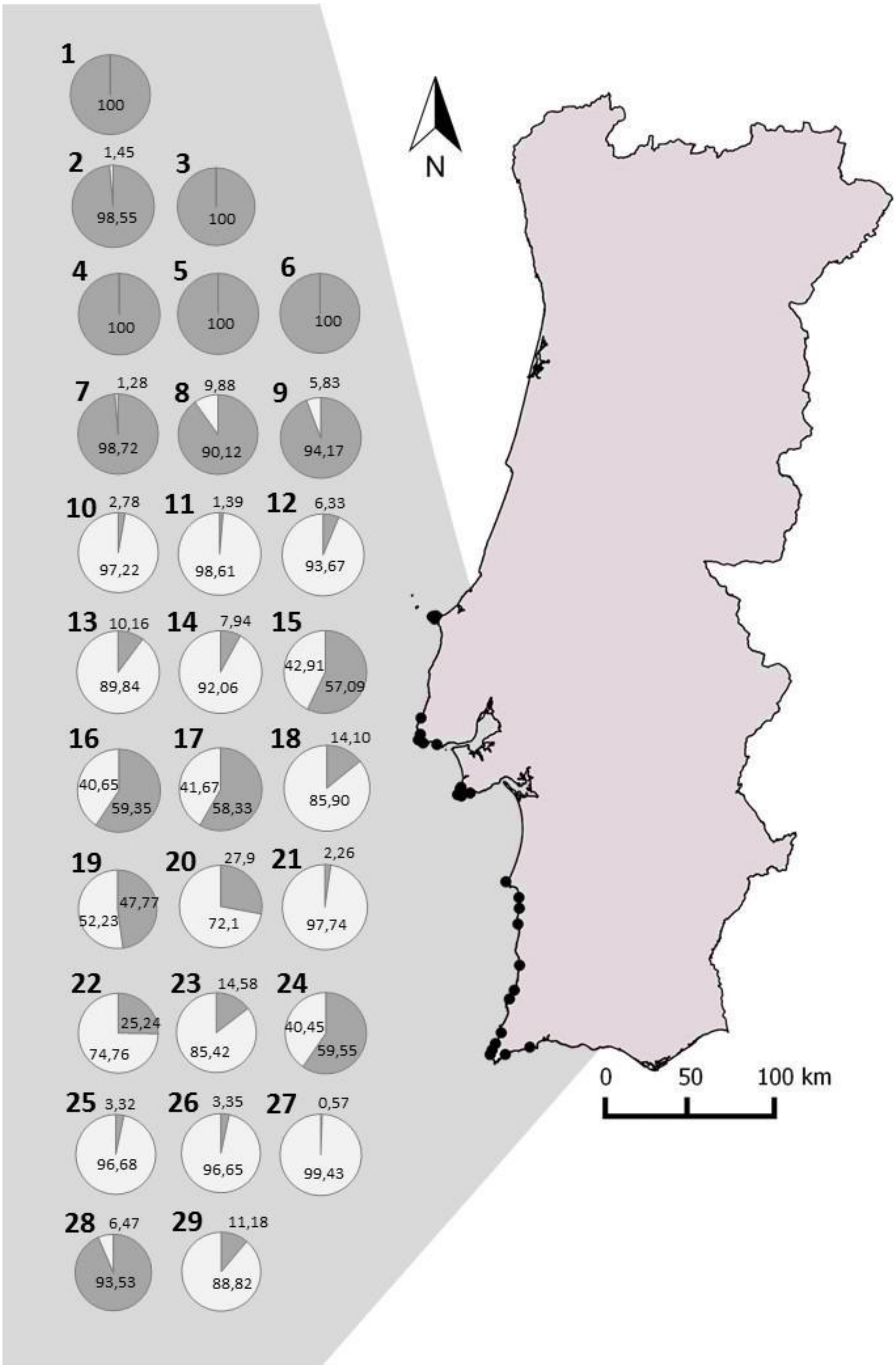


Fig. 5. Species relative abundance (%) along the Portuguese coast sampled: 1 - Papoa; 2- Portinho da Areia Norte East; 3 - Portinho da Areia Norte West; 4 - Cruz dos Remédios; 5 - Portinho da Areia Sul; 6 - Mexilhoeira; 7 - Maçãs; 8 - Cabo Raso; 9 - Avencas; 10 - Bicas; 11 - Foz; 12 - Lagosteiros North; 13 - Lagosteiros South; 14 - Porto da Baleeira; 15 - Ribeira do Cavalo; 16 - Pedras Amarelas; 17 - Burrinho; 18 - Queimado; 19 - Porto do Canal; 20 - Alteirinhos; 21 - Vale dos Homens North; 22 - Vale dos Homens South; 23 - Monte Clérigo; 24 - Amado; 25 - Cordoama; 26 - Ponta Ruiva; 27 - Telheiro; 28 - Ingrina; 29 - Luz. *P. lineatus* is represented in dark grey, and *P. sauciatus* in light grey.

Table V. *P. lineatus* and *P. sauciatus* mean shell height and width (±SD).

Local	*Phorcus lineatus*							*Phorcus sauciatus*						
	N	Mean Shell Height			Mean Shell Width			N	Mean Shell Height			Mean Shell Width		
Papoa	50	15.754	±	1.347	17.278	±	1.086	0						
Portinho da Areia Norte East	50	13.885	±	1.305	15.442	±	1.215	1	19.480	±	0.000	21.680	±	0.000
Portinho da Areia Norte West	41	18.155	±	3.238	19.772	±	2.943	0						
Cruz dos Remédios	9	22.510	±	7.181	22.251	±	5.455	0						
Portinho da Areia Sul	1	16.820	±	0.000	17.730	±	0.000	0						
Mexilhoeira	50	13.081	±	0.599	15.768	±	0.445	0						
Maçãs	50	13.355	±	1.235	16.063	±	1.035	1	9.890	±	0.000	13.090	±	0.000
Cabo Raso	50	12.290	±	0.737	14.600	±	0.534	41	12.826	±	2.058	16.568	±	1.869
Avencas	50	13.553	±	1.681	15.068	±	1.319	13	15.131	±	3.739	17.631	±	3.300
Bicas	3	13.937	±	2.023	14.417	±	1.846	50	10.662	±	0.937	13.482	±	1.024
Foz	3	14.327	±	2.099	16.017	±	1.263	50	11.986	±	0.711	15.847	±	0.494
Lagosteiros North	24	14.671	±	2.227	15.922	±	1.957	0	12.110	±	0.681	15.926	±	0.565
Lagosteiros South	13	17.077	±	2.029	17.822	±	1.370	50	12.173	±	0.848	16.626	±	0.662
Porto da Baleeira	30	14.073	±	2.515	15.308	±	2.247	50	10.945	±	0.807	14.531	±	0.554
Ribeira do Cavalo	50	13.696	±	0.914	15.242	±	0.875	0	9.799	±	0.818	13.052	±	0.843
Pedras Amarelas	50	15.009	±	1.136	16.454	±	0.837	50	13.189	±	2.708	16.801	±	2.948
Burrinho	50	15.154	±	1.589	15.780	±	1.455	50	11.738	±	2.859	14.580	±	3.069
Queimado	0	12.863	±	2.312	14.028	±	2.215	50	13.125	±	0.875	15.959	±	0.539
Porto do Canal	50	13.077	±	0.742	14.219	±	0.527	0	12.079	±	0.814	14.752	±	0.637
Alteirinhos	50	14.392	±	1.285	15.751	±	1.172	50	13.668	±	0.950	16.394	±	0.739
Vale dos Homens North	12	11.384	±	1.700	13.127	±	1.958	50	11.280	±	0.972	14.496	±	0.586
Vale dos Homens South	50	12.365	±	1.400	14.070	±	1.230	50	11.704	±	0.880	14.574	±	0.550
Monte Clérigo	50	12.370	±	0.798	14.399	±	0.581	50	11.755	±	0.811	15.152	±	0.599
Amado	50	15.003	±	0.700	17.055	±	0.602	50	12.897	±	0.970	16.312	±	0.609
Cordoama	19	14.179	±	1.744	15.988	±	1.663	50	12.367	±	1.493	15.741	±	1.509
Ponta Ruiva	27	13.459	±	2.489	15.750	±	2.447	50	11.352	±	1.154	15.527	±	1.023
Telheiro	1	23.460	±	0.000	23.120	±	0.000	50	11.601	±	0.883	15.287	±	0.715
Ingrina	50	13.201	±	0.764	14.374	±	0.485	24	11.811	±	1.442	15.101	±	1.655
Luz	50	15.025	±	1.715	16.695	±	1.617	50	11.233	±	0.584	14.005	±	0.513
TOTAL	983	14.763	±	1.638	16.190	±	1.392	880	12.283	±	1.166	15.546	±	1.042

It is important to refer that, when there were enough individuals in the sample for the geographic morphometric study, only the specimens with approximately the same size were chosen, i.e. between 13 mm and 17 mm (width) in order to facilitate comparisons. This means that regardless the presence of larger or smaller individuals in the sample, they were not considered in this study. On the other hand, when there were less than 50 individuals, they were all considered, independently of their size, thus creating a greater SD in the measurements performed. This was necessary to maintain an adequate number of specimens from each location to allow robust statistics.

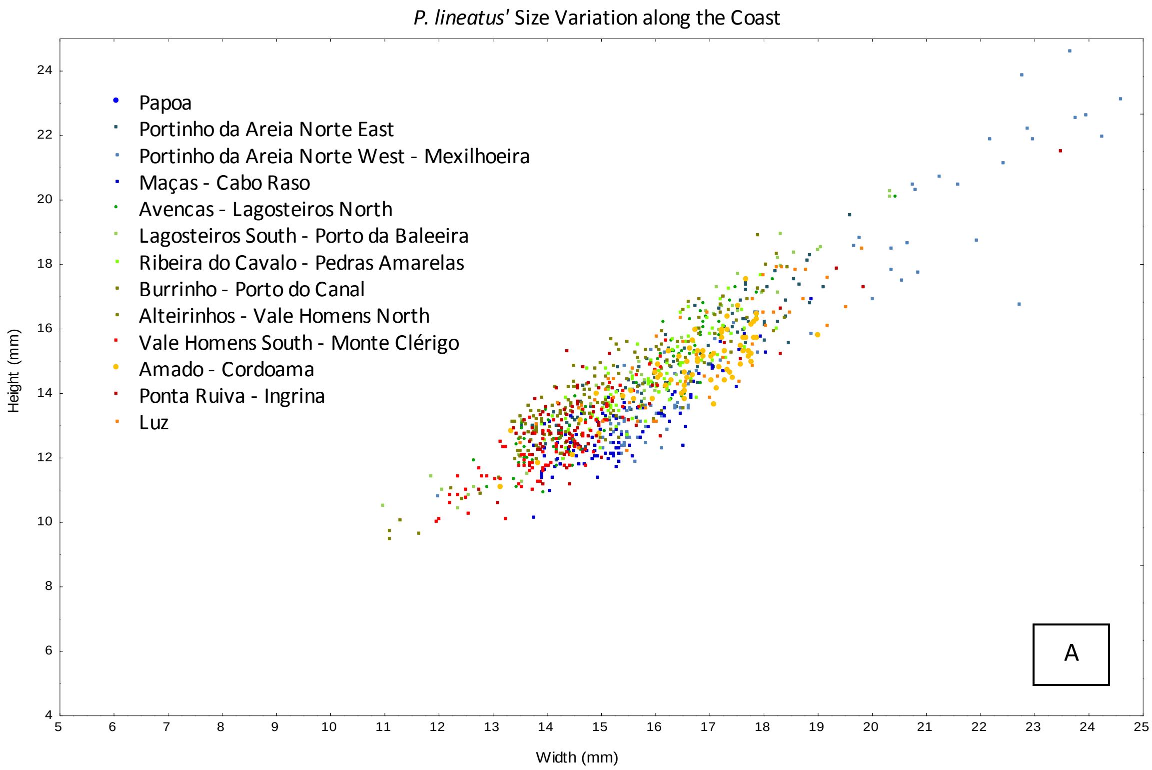
2.1. Geographic Morphometric Study

Size Variation along the Coast

P. lineatus results from the ANCOVA (

Fig. **6**A) indicated that there were significant differences of width/height variation between populations along the coast ($F = 176.851$; $p < 0.0001$). The northern populations (from Papoa to Cabo Raso, represented in blue) have bigger width and height, and the central ones (represented in tones of green) have a smaller size. Southern populations (from Vale dos Homens North to Luz, represented in warm colours, i.e. orange and red tones) are grouped in a lower range of width/height, contrary to populations from the central and northern region, where there is a higher variability. In *P. sauciatus,* the ANCOVA (

Fig. **6**B) also indicated that there were significant differences of width/height variation between populations along the coast ($F = 193.001$; $p < 0.0001$). However, there is not a clear pattern.



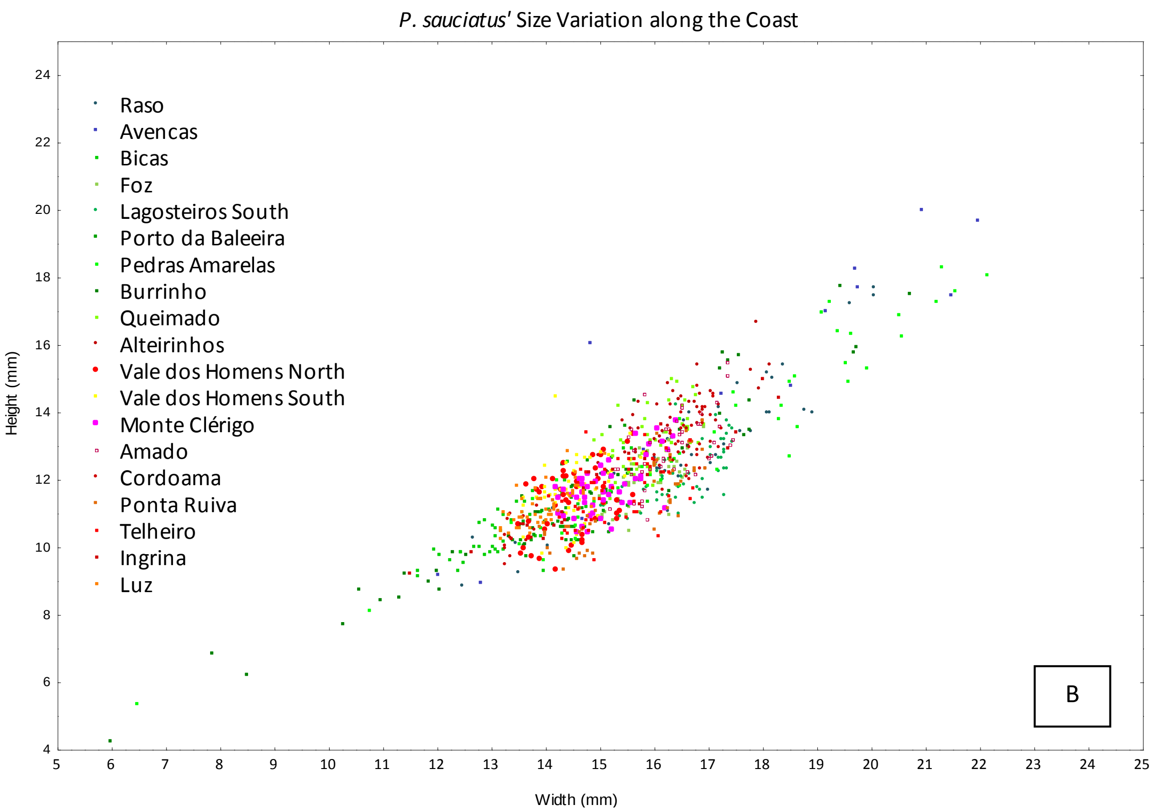


Fig. 6. Variation of shell width and shell height of *P. lineatus* (A) and *P. sauciatus* (B) along the coast, in all populations except the ones with less than ten individuals.

Wave Exposure

◆ Integrated effects of shore orientation

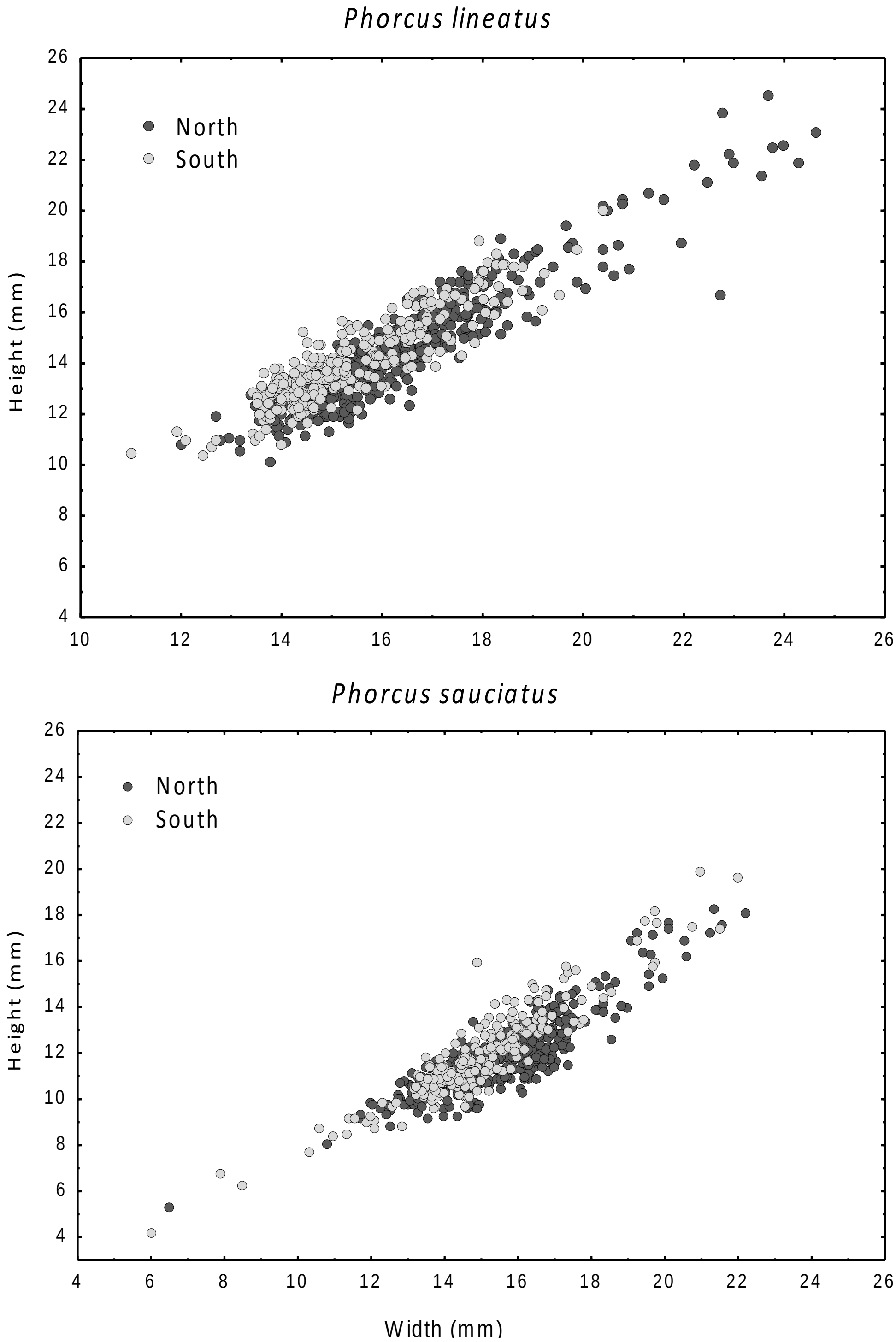


Fig. 7. Relation of shell height (mm) with shell width (mm) of *P. lineatus* (A) and *P. sauciatus* (B) specimens, considering all specimens for the north of capes, and all specimens of the south of capes.

Results from the ANCOVA (Fig. 7) showed that there were significant width/height differences among populations in both species ($F = 1807.085$, $p < 0.0001$ in *P. lineatus* and $F = 1284.531$, $p < 0.0001$ in *P. sauciatus*). Although similar when comparing North and South populations, *P. sauciatus* is clearly more flattened than *P. lineatus* (Fig. 8).

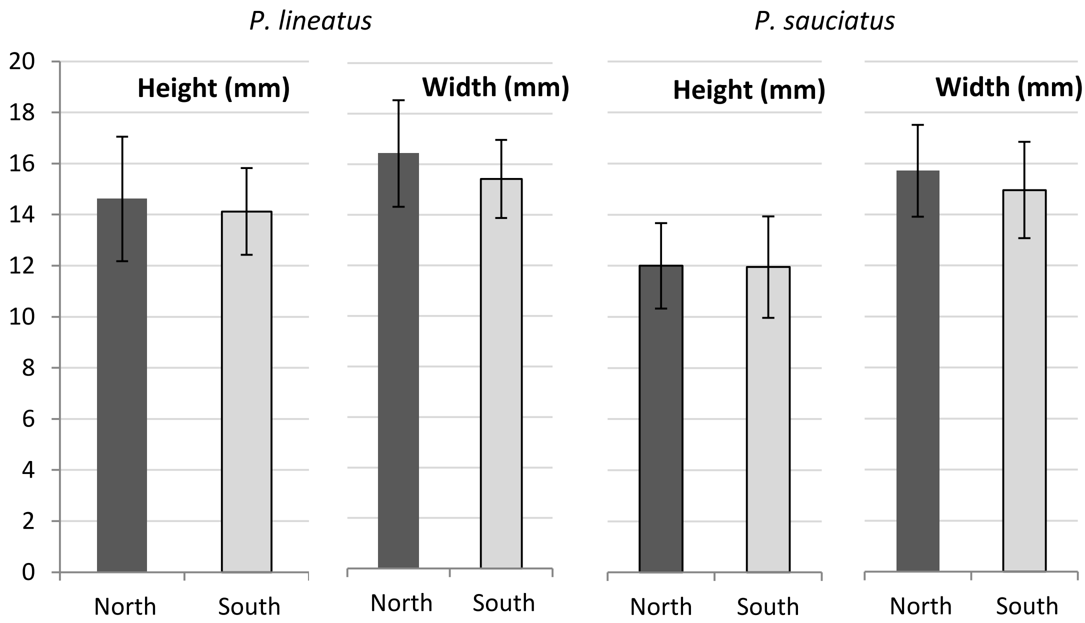


Fig. 8. Mean and Standard Deviation of shell height (mm) and shell width (mm) of specimens considering the integrated effects of shore orientation. All populations located in the north of capes were grouped together, as well as populations located in the south of capes.

◆Effects of orientation in each Cape

For both species, results from the ANCOVA (Fig 9 for *P. lineatus* and Fig. 10 for *P. sauciatus*) showed that there were significant width/height differences between northern and southern populations from each cape (Table VI). In general, *P. lineatus* (Fig. 9) populations from the northern side of the capes are less high than the populations from the southern side, except in Cape Espichel, where apparently all populations seem similar (Fig. 9B). Regarding *P. sauciatus* (Fig. 10) that pattern is less evident, except in Cape of São Vicente (Fig. 10D).

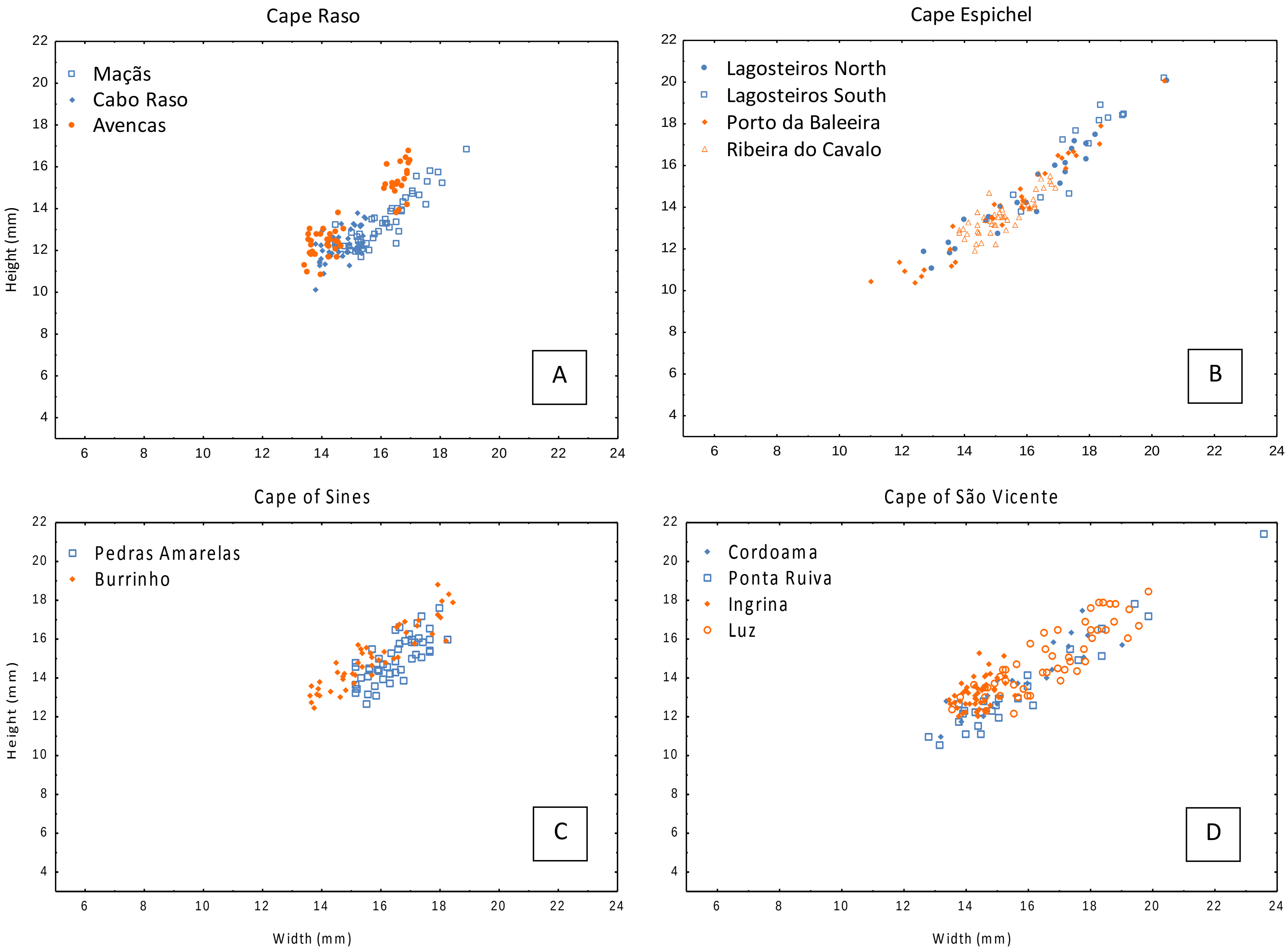


Fig. 9. Results from the ANCOVA on the variation of width and height of shells, comparing Northern (blue) and Southern (orange) populations of *P. lineatus*; A - Cape Raso; B - Cape Espichel; C - Cape of Sines; D - Cape of São Vicente.

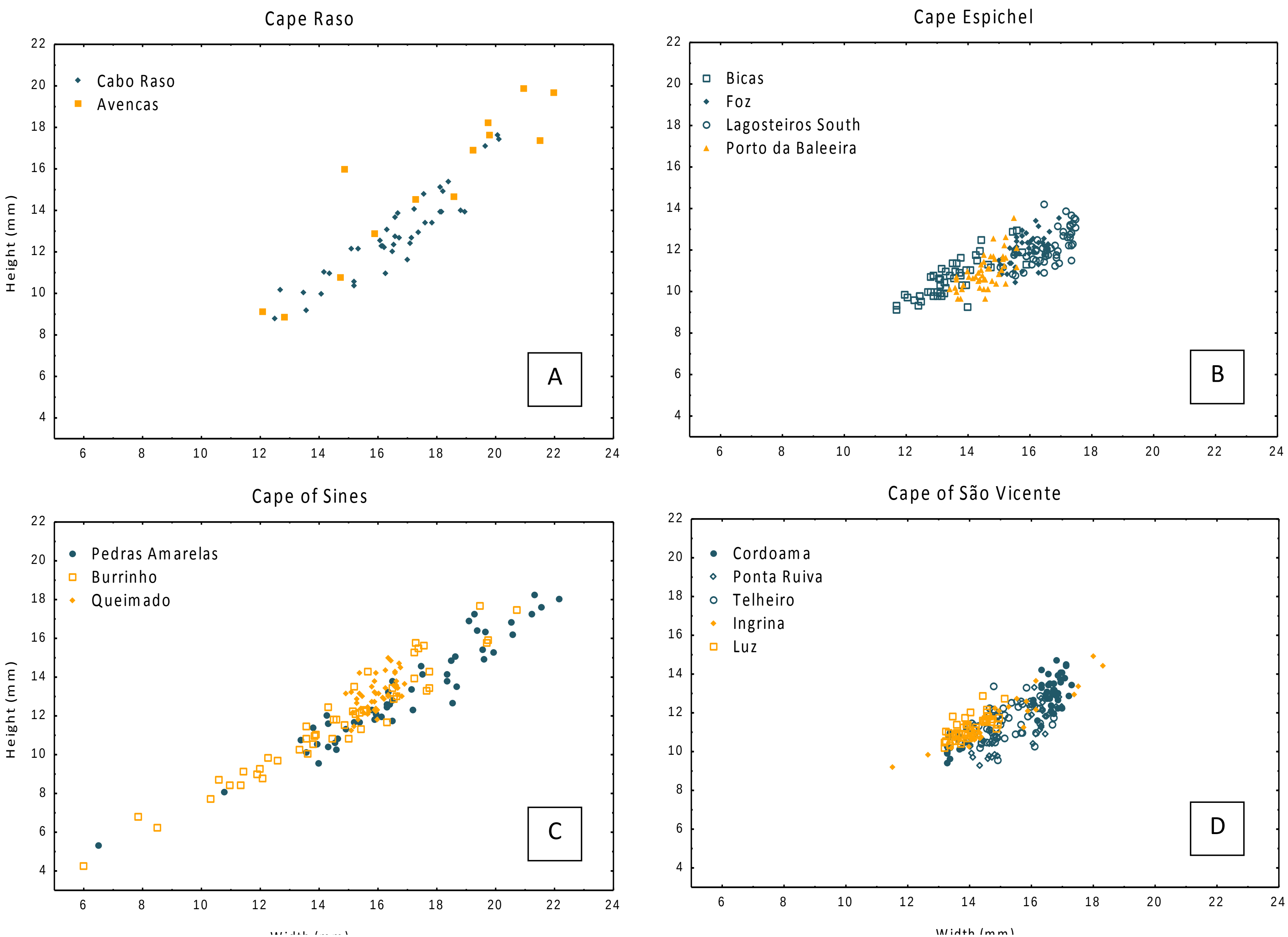


Fig. 10. Results from the ANCOVA on the variation of width and height of shells, comparing Northern (blue) and Southern (orange) populations of *P. sauciatus*; A - Cape Raso; B - Cape Espichel; C - Cape of Sines; D - Cape of São Vicente.

Table VI. Results from the ANCOVA on width and height for *P. lineatus* (left) and *P. sauciatus* (right), comparing locations from the North with locations from the South of each cape.

	Cape	F	p
Phorcus lineatus	Raso	239.161	< 0,001
	Espichel	516.566	< 0,001
	Sines	159.492	< 0,001
	São Vicente	305.292	< 0,001

	Cape	F	p
Phorcus sauciatus	Raso	139.473	< 0,001
	Espichel	186.758	< 0,001
	Sines	660.340	< 0,001
	São Vicente	210.678	< 0,001

◆ **<u>Short scale - two sides in the same beach location</u>**

For both species, the ANCOVA performed on width and height indicated that (Fig. 11) there were significant differences between populations from from the two sites in the same location (Table VII) indicates results of the ANCOVA for *P. lineatus;* for *P. sauciatus* $F = 10.022$; $p < 0.0001$).

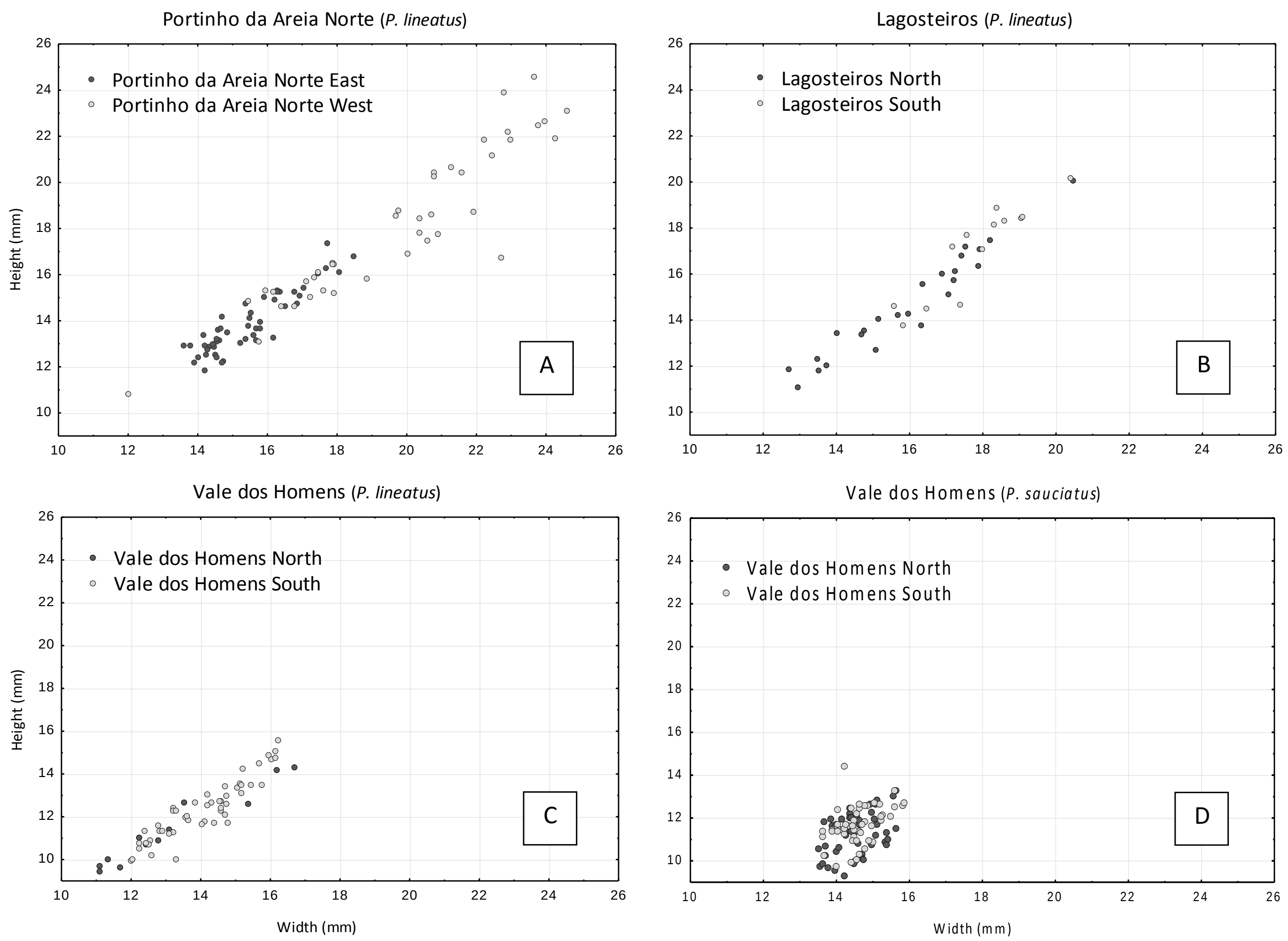


Fig. 11. Locations with two sampling sites were analysed for width/height variation with exposure. *P. lineatus*: A - Portinho da Areia Norte East and West; B - Lagosteiros North and South; C - Vale dos Homens North and South; *P. sauciatus:* D - Vale dos Homens North and South. Sampling sites located on the North or East side of the beaches are represented in black and the ones from the South or West in grey.

Table VII. Results from the ANCOVA performed on the on width/height for *P. lineatus*, comparing two sampling sites from the same beach location.

	Location	F	p
Phorcus lineatus	Portinho da Areia Norte East // Portinho da Areia Norte West	246.175	< 0,001
	Lagosteiros North // Lagosteiros South	180.964	< 0,001
	Vale dos Homens North // Vale dos Homens South	205.542	< 0,001

2.2. Ontogenetic Morphometric Study

Results from the ANCOVA (Fig. 12 for *P. lineatus* and Fig. 13 for *P. sauciatus*) indicated that there were significant width/height differences among the different size classes in both species (F = 96.213, p < 0.0001 in *P. lineatus* and F = 69.329; p < 0.0001 in *P. sauciatus*). All size classes where significantly different from each other (*p-values* for Tukey HSD tests where all < 0.0001).

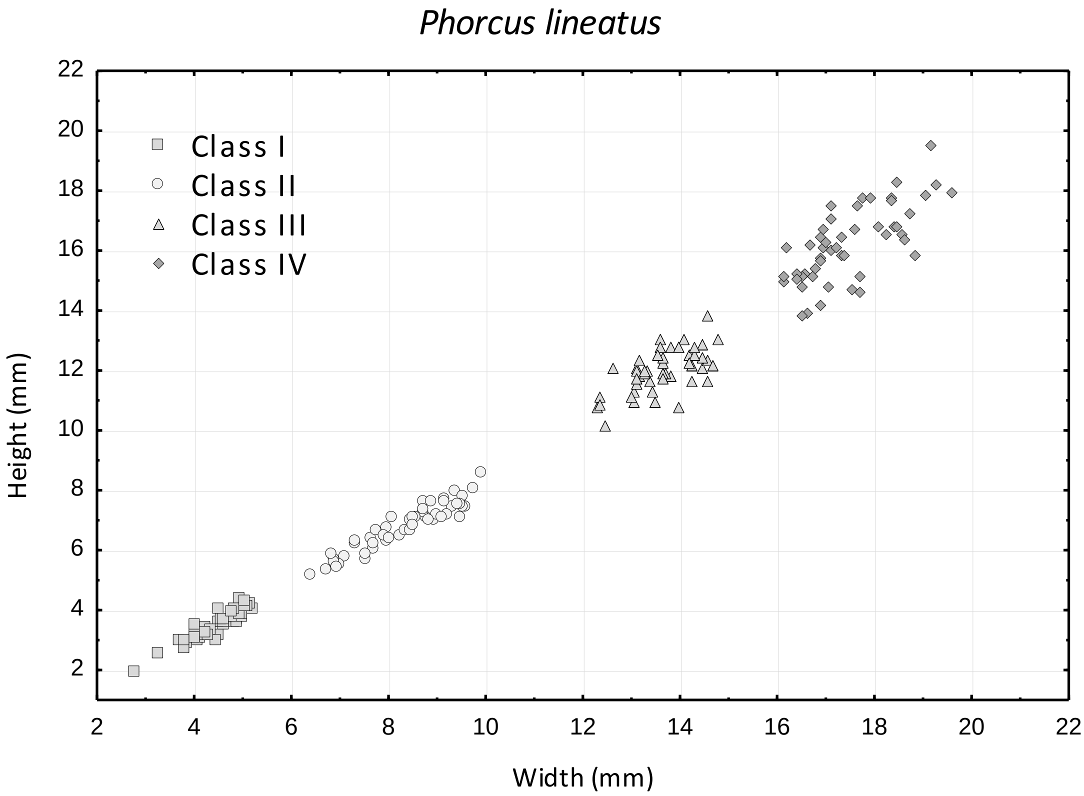


Fig. 12. Width and height variation of *P. lineatus'* population from Avencas. Each symbol represents a different size class, corresponding to different age classes: (I- [1 mm - 5 mm], II- [6 mm - 10 mm], III- [11 mm - 15 mm], and IV - [16 mm - 20 mm].

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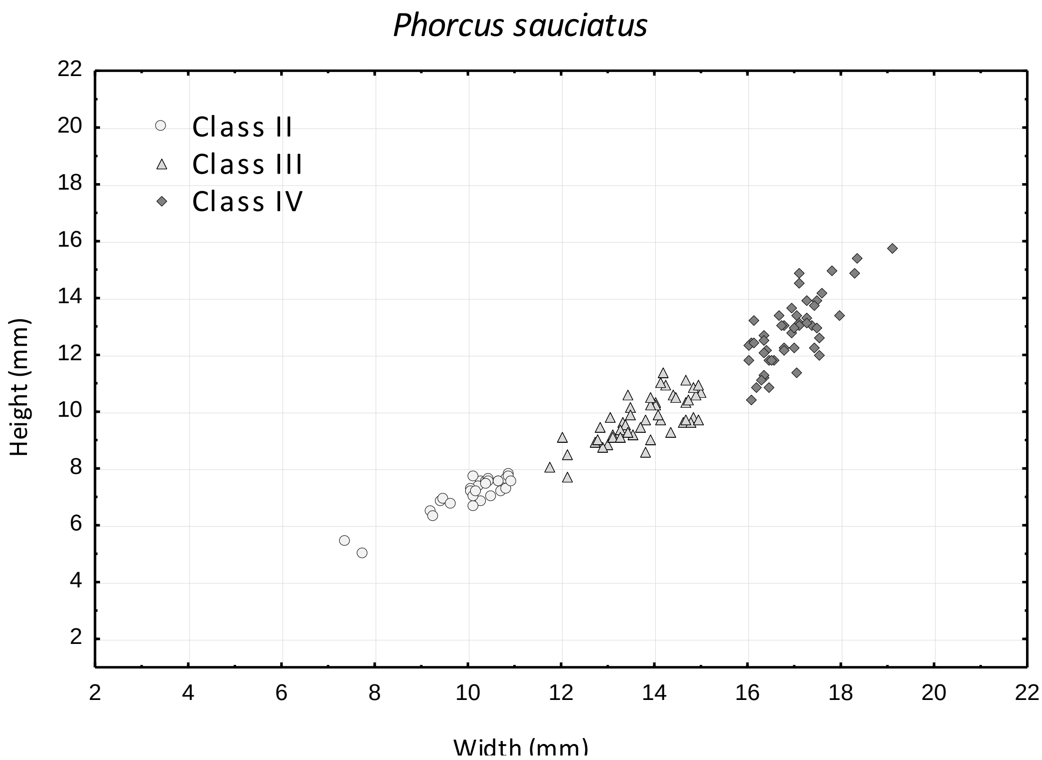


Fig. 13. Width and height variation of *P. sauciatus'* population from Ponta Ruiva. Each symbol represents a different size class, corresponding to different age classes: (II- [6 mm - 10 mm], III- [11 mm - 15 mm], and IV - [16 mm - 20 mm].

3. Morphometry - Geometric Approach

The analysis of geographic morphometric variation and the analysis of ontogeny morphometric variation were preformed separately, thus the output from the morphometric shape analysis software (tpsRelw 1.49) was composed by four data groups: two for the ontogeny study (one for each species) and two for the geographic study (again, one for each species).

The shape analysis approach revealed the existence of morphological variations in the shells, both between locations and between size classes.

3.1. Geographic Morphometric Study

In *P. lineatus* 66.11% of the variance was explained by the first three Relative Warps (RWs) - RW1 accounted for 34.49 %, RW2 for 20.27% and RW3 explained 11.35% of the variance. In *P. sauciatus* the percentage of variance explained by the first three RWs was 70.36%. The first RW accounted for 38.79% of the explained variance, the second one explained 20.05% and the third one explained 11.51% of the variance.

The pattern of shape variation along the first two RWs is shown in Fig. 14. Along the RW1, and for both species, the shape variation is characterized by a progressive narrowing of the shell width, accompanied by a relative higher spire and a diminishing lateral projection of the aperture. At the negative end of this axis (A), specimens have an approximately rhombular shape, with a wide aperture, and a short, but well-marked, tooth; whereas in the positive end (B) the specimens are slightly higher and narrower, resulting in triangular shape, with a smaller aperture, and with a longer and only slightly marked tooth. Fig. 14 also shows shape change along the second axis: there is an increase not only of the width of the shell, but also in the aperture's size. There is also a progressive flattening of the columela tooth (it becomes less prominent). At the positive end of this axis (C), specimens are higher, with a flattened aperture and a short, but well-marked, tooth. Whereas in the negative end (D) the shell aperture is much wider, the tooth is longer and only slightly marked.

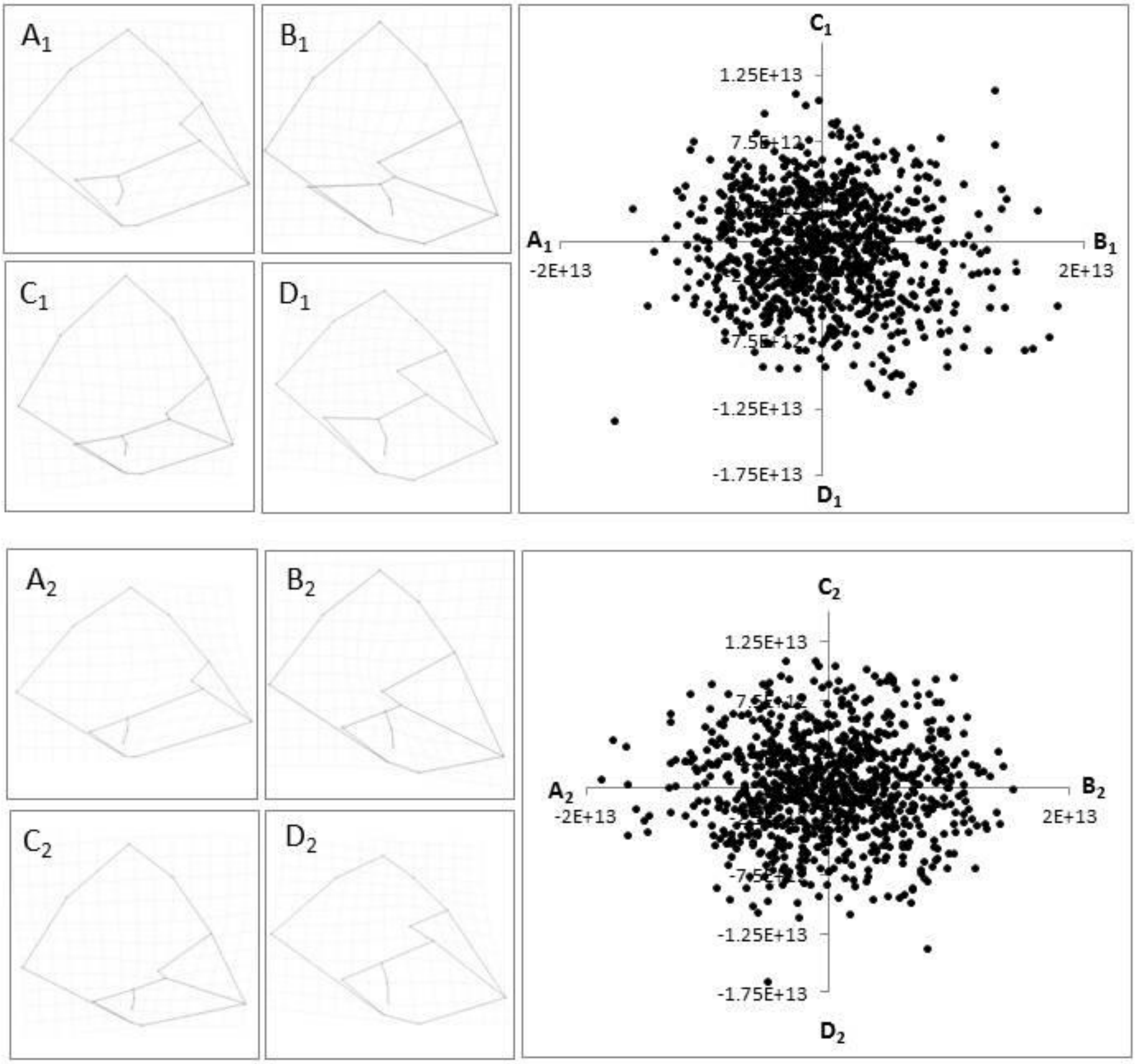


Fig. 14. Spline grids showing shape deformation along Relative Warps 1 and 2 of the shell of *P. lineatus* (above) and *P. sauciatus* (down). The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change.

Shape Variation along the Coast

Phorcus lineatus

Results from the nested MANOVA (Fig. 15A) revealed significant shape differences among studied populations (Wilks' $\lambda = 0.3400$; $F = 18.5099$; $p < 0.0001$). Although the post-hoc Tukey HSD confirmed this possibility, not all populations were significantly different from each other.

Lagosteiros North was significantly different from all populations except from Lagosteiros South and Ponta Ruiva. On the other hand, Baleeira was only significantly different from two populations: Cabo Raso and Lagosteiros North.

Phorcus sauciatus

Results from the nested MANOVA (Fig. 15B) showed that there were significant shape differences among populations (Wilks' $\lambda = 0.2855$; $F = 24.739$; $p < 0.0001$). Not all populations varied their shell shape significantly from each other. Burrinho and Alteirinhos were the populations more significantly different from all the others. On the contrary, Avencas and Monte Clérigo were only significantly different from Burrinho, and from Burrinho and Alteirinhos, respectively.

Although the ellipses from different populations for both species vary in their shape, the respective centroids are very close to each other (Fig. 15), especially for *P. lineatus*. For *P. sauciatus* some of the populations are deviated from the centre, especially Alteirinhos, which is almost only in the positive side of the axis RW1.

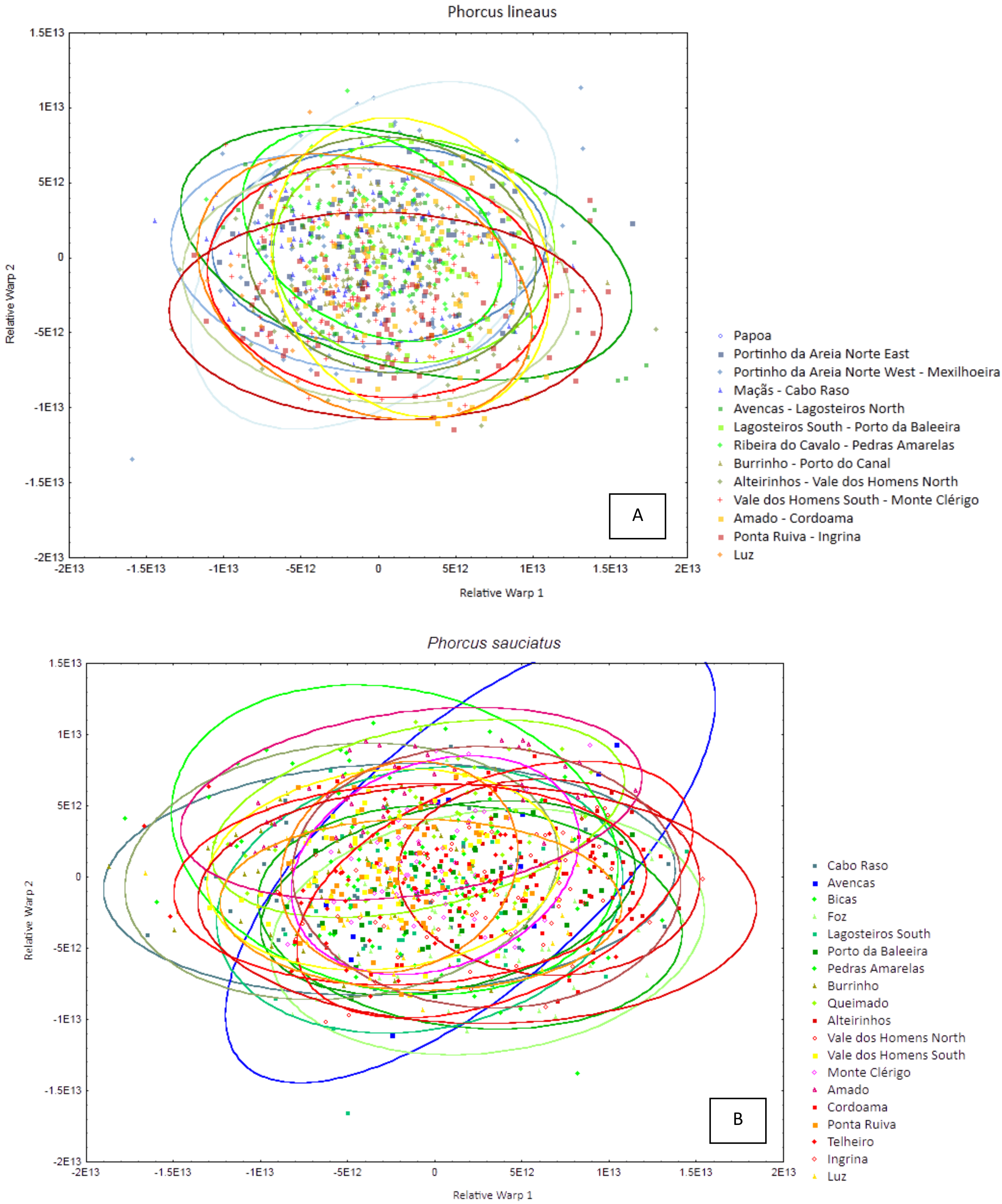


Fig. 15. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits. *P. lineatus* (A) and *P. sauciatus* (B).

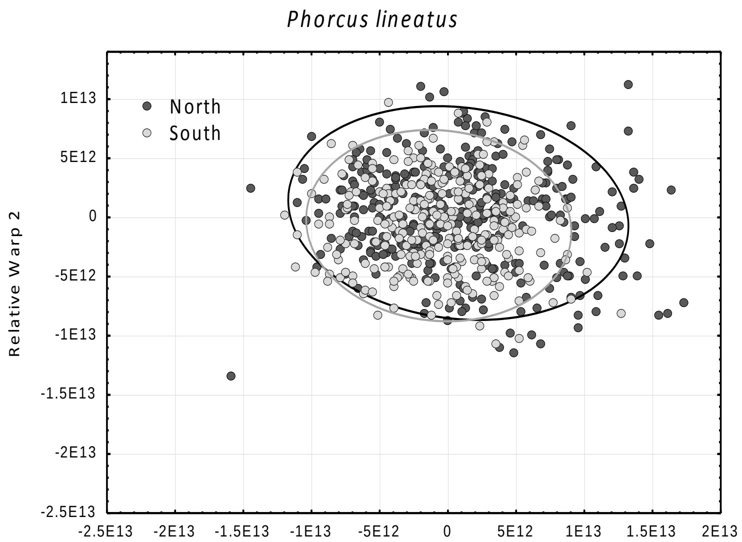
Wave Exposure

◆ Integrated effects of shore orientation

For both species, results from the nested MANOVA (*P. lineatus*

Fig. 16A, *P. sauciatus*

Fig. 16B) indicated significant shape differences between the populations from the Northern and Southern sides of the Portuguese capes (Wilks' $\lambda = 0.781$; $F = 69.767$; $p < 0.0001$ in *P. lineatus* and Wilks' $\lambda = 0.969$; $F = 6.663$; $p < 0.0001$ in *P. sauciatus*).



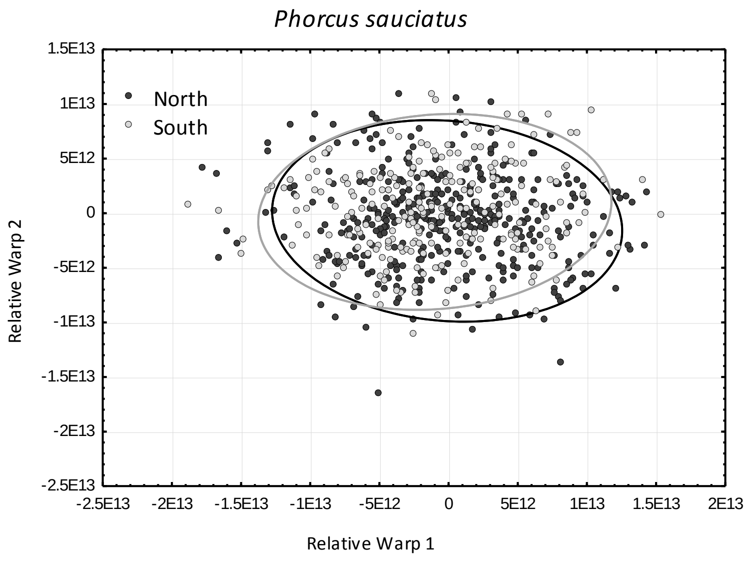


Fig. 16. Variation of shell shape of *P. lineatus* (A) and *P. sauciatus* (B). The populations from the Northern side of the capes are represented in blue, and the ones from the Southern side are represented in orange. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits

◆Effects of orientation in each Cape

Phorcus lineatus

Results from the nested MANOVA (Fig. 17) indicated that there were significant differences of shell shape between Northern and Southern populations (Table VIII).

Table VIII. Results from the nested MANOVA

	Cape	wilk's value	F	p
Phorcus lineatus	Raso	0.679	23.035	0.000
	Espichel	0.629	22.169	0.000
	Sines	0.877	4.493	0.005
	São Vicente	0.546	39.414	0.000

Porto da Baleeira and Ribeira do Cavalo (in Cape Espichel area) were significantly different from populations north from the cape (Lagosteiros North and South). The same happened in Cape of São Vicente, where Ingrina was significantly different from the all populations on the north of the cape. Both Cape Raso and Portinho da Areia Norte East were significantly different from all the locations nearby.

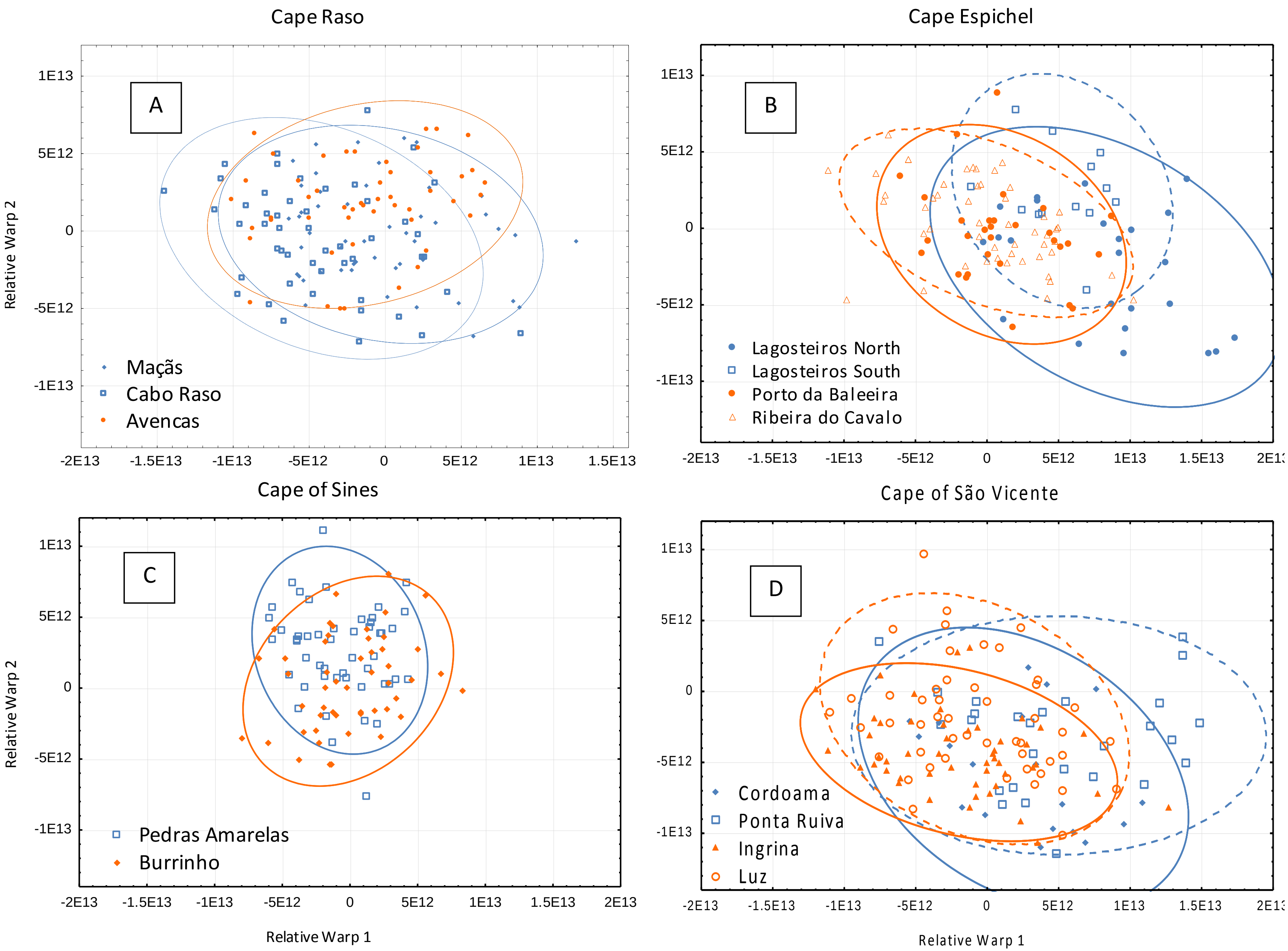


Fig. 17. Variation of shell shape of *P. lineatus* comparing locations from the North with locations from the South of each cape. 1 - Cape Raso; 2 - Cape Espichel; 3 - Cape of Sines; 4 - Cape of São Vicente. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits.

Phorcus sauciatus

Results from the nested MANOVA (Fig. 18) indicated that there were significant differences of shell shape between northern and southern populations (Table IX).

Table IX. Results from the nested MANOVA

	Cape	wilk's value	F	p
Phorcus sauciatus	Raso	0.862	7.814	0.000
	Espichel	0.751	21.662	0.000
	Sines	0.861	7.814	0.000
	São Vicente	0.588	51.433	0.000

In Cape Espichel, Lagosteiros South was significantly different from the Northern populations, Bicas and Foz. On the other hand, Porto da Baleeira showed no significantly differences to other populations. In Cabo de São Vicente, Cordoama was significantly different from the all populations except from Ingrina.

Moreover, only Cordoama was significantly different from Luz. In Cape of Sines, Burrinho was significantly different from the other two populations.

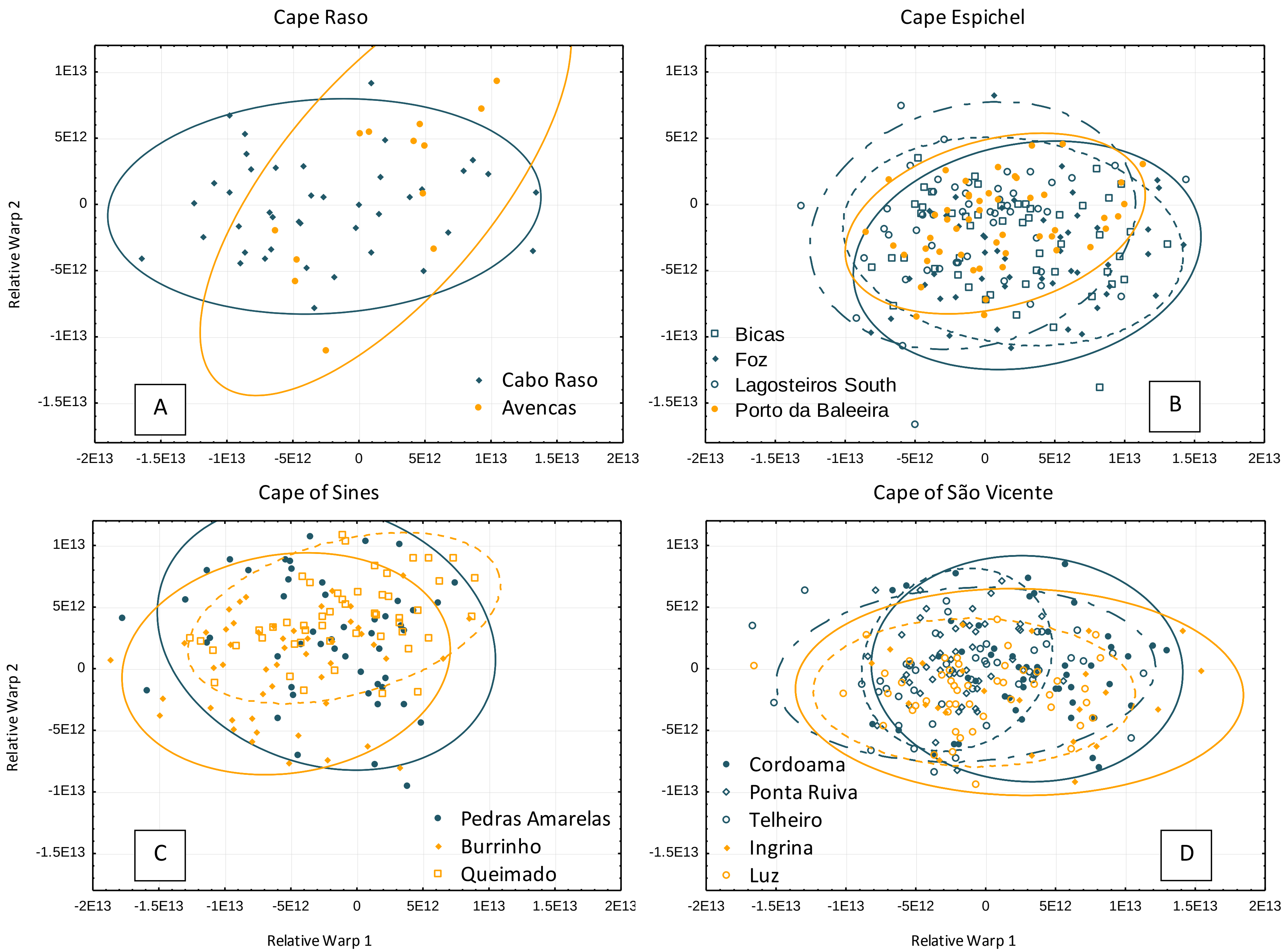


Fig. 18. Variation of shell shape of *P. sauciatus* comparing locations from the North with locations from the South of each cape. 1 - Cape Raso; 2 - Cape Espichel; 3 - Cape of Sines; 4 - Cape of São Vicente. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits.

◆ Short scale - two sides in the same beach location

Phorcus lineatus

Results from the nested MANOVA (Fig. 19) revealed significant differences between populations from the two sides (

Table X) within the same beach.

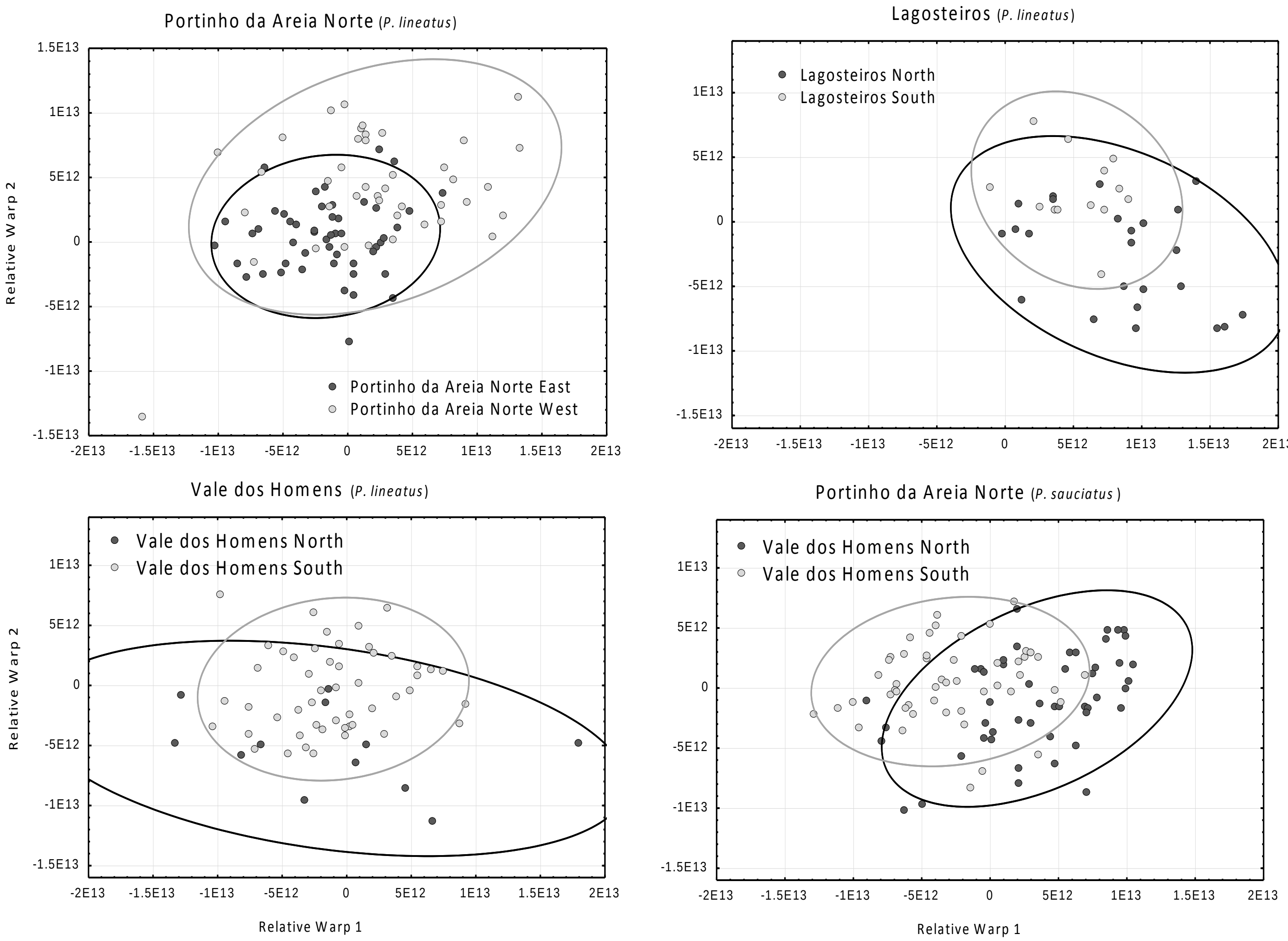


Fig. 19. Locations with two sampling sites - one exposed and the other sheltered - were analysed for shape variation with exposure. *P. lineatus*: A - Portinho da Areia Norte East and West; B - Lagosteiros North and South; C - Vale dos Homens North and South; *P. sauciatus:* D - Vale dos Homens North and South. Sampling sites located on the North or East side of the beaches are represented in black and the ones from the South or West in grey. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits.

Table X. Results from the nested MANOVA

	Location	wilk's value	F	p
P. lineatus	Portinho Areia Norte East // Portinho Areia Norte West	0.474	32.217	0.000
	Lagosteiros North // Lagosteiros South	0.500	11.018	0.000
	Vale Homens North // Vale Homens South	0.590	22.193	0.000

Phorcus sauciatus

Results from the nested MANOVA (Fig. 19) showed that there were significant differences between populations within the same beach location (Wilks' λ = 0.590; F = 22. 193; p < 0.0001).

3.2. Ontogenetic Morphometric Study

In *P. lineatus* the percentage of variance explained by the first three RWs was 73.14%. The first relative warp (RW1) accounted for 45.61% of the explained variance, the second relative warp (RW2) explained 19.82% and the third one (RW3) only explained 7.71%. Regarding *P. sauciatus*, the RW1 accounted for 37.46 %, the RW2 for 30.20% and the last one (RW3) explained only 7.44% of the variance, making a total of 75.09%.

The pattern of shape variation along the first two RWs in *P. lineatus* is shown in Fig. 20A. In this species the first axis revealed shape differences manly at the shell's aperture level, in the distances between LM 5 and LM 6 and consequently between LM 7 and LM 12. At the negative end of this axis, specimens have an approximately rhombular shape, with longer and only slightly marked tooth. Whereas in the positive end the specimens are slightly higher and narrower, resulting in triangular shape, with smaller apertures and a short, but well-marked, tooth. The second axis revealed shape differences manly on the shell's height. This axis also revealed shape differences on the size and shape of the tooth. At the positive end of this axis, specimens are flattened, almost without a distinct tooth, whereas in the negative end specimens are higher, presenting a marked, short and curvy tooth.

In Fig. 20B is represented the pattern of shape variation along the first two RWs in *Phorcus sauciatus*. Along the first axis there was a variation in the width and flattening of the shell, the larger the relative warp, the more flattened and broader is the shell. There was also an increasing of the aperture's size, and a decrease of the distance between LM 2 and LM 3 (landmarks placed in the suture of the last/second whorl), which translates to a smaller whorl. Finally there was also an increase of the distance between LM 5 and LM 6 and consequently between LM 7 and LM 12. Regarding the second axis, there was an increase of the distance between LM 5 and LM 6 and consequently a decrease of the distance between LM 7 and LM 12. There was also a slightly flattening of the shell.

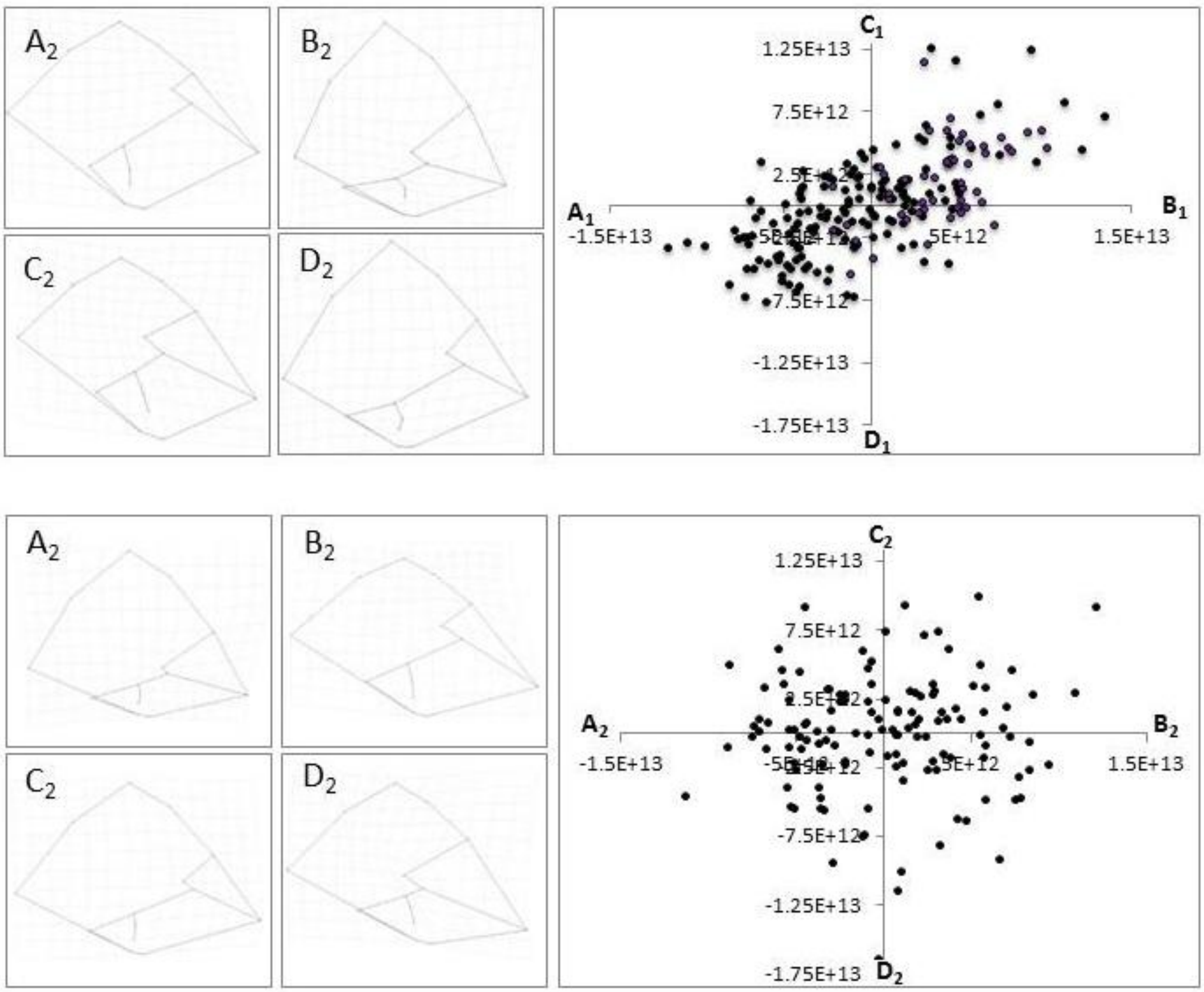


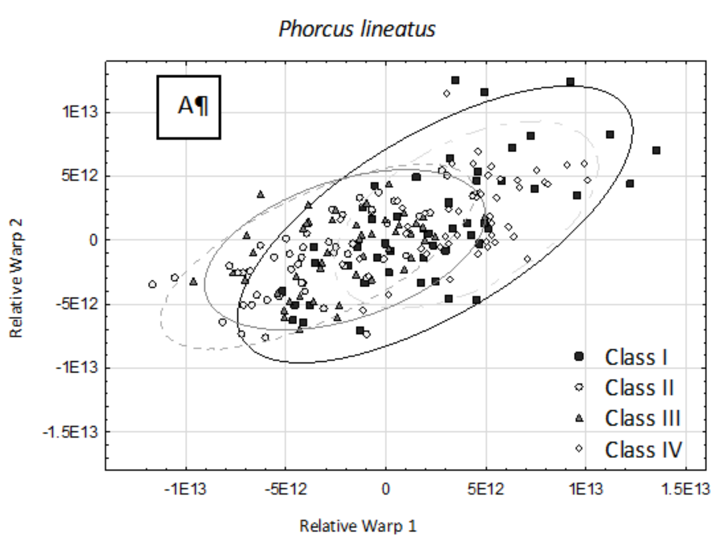
Fig. 20. Spline grids showing shape deformation along Relative Warps 1 and 2 of the shell of *P. lineatus* (up) and *P. sauciatus* (down). The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits.

Phorcus lineatus

Results from the nested MANOVA (**Error! Reference source not found.**A) showed that there ere significant differences between size classes (Wilks' λ = 0.533; F = 15.48023; p < 0.0001). Although the post-hoc Tukey HSD confirmed this possibility, not all size classes were significantly different from each other. The differences between size classes 1 [1 mm - 5mm] and 4 [16 mm - 20 mm], and between size classes 2 [6 mm - 10 mm] and 3 [11 mm - 15 mm] are not significantly relevant, which means that the shell shape within these two groups is similar.

Phorcus sauciatus

Results from the nested MANOVA (**Error! Reference source not found.**B) showed that there ere significant differences between size classes (Wilks' λ = 0.546; F = 14.7128; p < 0.0001). The post-hoc Tukey HSD tests confirmed that all size classes were significantly different from each other.



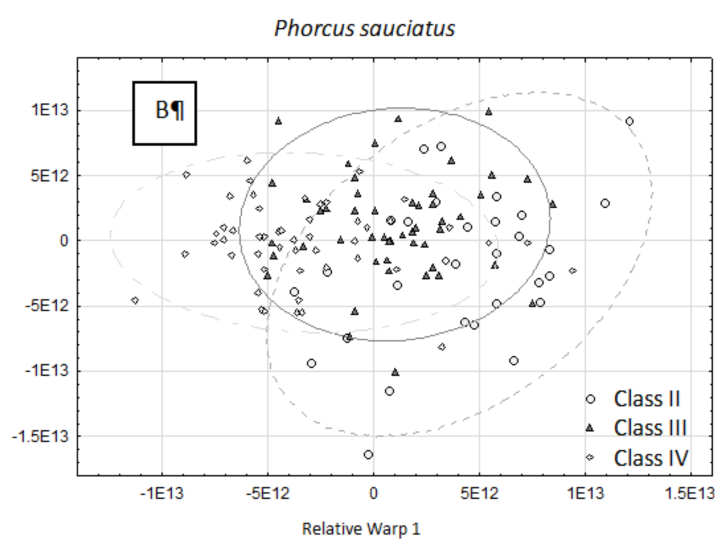


Fig. 21. The graph illustrates the specimens' distances to the mean configuration of the shell (graph origin 0.0). Each point in the scatterplot represents a unique shape and the axes represent vectors of shape change. The ellipses correspond to a probability of 90% of the points being distributed within their limits. (A - *P. lineatus* and B - *P. sauciatus*)

DISCUSSION

(1) How does the abundance of these two species vary along the Portuguese coast?

The observed distribution of the two species was as expected (Crothers 2001; Kendall 1987; Pedro 2004; Donald et al. 2012; Mieszkowska 2005; Rubal et al. 2014). *P. lineatus*, the northern species, was more abundant in the north, whereas *P. sauciatus*, the southern species, was more abundant in the South.

Sea temperature exerts a strong control over species' distributions (Sorte et al., 2011), and over the timing and success of reproduction within populations (Kendall 1987; Mieszkowska et al. 2007). It also influences across geographic ranges for marine intertidal invertebrates (Mieszkowska et al. 2007; Mieszkowska et al. 2013). According to Crothers' (1998) study in Somerset (UK), hot summers improved the recruitment of *P. lineatus*. On the other hand, low temperatures affect negatively the recruitment. In places with coastal upwelling, and consequently low Sea Surface Temperature (SST) (Fiúza et al. 1982; Raffaeli & Hawkins1996; Boaventura et al. 2002; Lemos & Pires 2004), it can be expected a reduced recruitment of the subtropical *P. sauciatus*. The North Portuguese coast does have a low SST (Fiúza et al. 1982; Boaventura et al. 2002), which may be limiting *P. sauciatus* recruitment, thus explaining the lower densities of the species in North Portugal (Rubal et al. 2014).

Despite the reduced numbers, *P. sauciatus* indeed inhabits the northern Portuguese coast. Its presence may be linked to the weakening of the upwelling since the 1940s that has led to an increase of +0.01 ^{o} C per year in the coastal SST (Lemos & Pires 2004). This gradual increase in SST in North Portugal could be the responsible for the recent colonization by *P. sauciatus* (Rubal et al. 2014). Many studies report an increase in the abundance and range of expansion, to the north, for subtropical species (Lima et al. 2006; Rubal et al. 2013). Similarly, in the British islands, studies also have shown poleward range shifts of warm-water species in relation to SST increase (Hawkins et al. 2003; Mieszkowska et al. 2006; Mieszkowska et al. 2007).

From the Tejo River to the South, *P. sauciatus* clearly has a higher abundance that *P. lineatus*. The Ingrina beach was an exception to this expected distribution. Although it lies in the south Portuguese coast, *P. lineatus* was more abundant than *P. sauciatus*. One possible reason for this outlier is the degree of exposure in this beach, a drive force that influences the structure of rocky intertidal communities (Ballantine 1961; Lewis 1964; Mieszkowska et al. 2013). Many authors propose that *P. sauciatus* is more frequent at exposed shores and *P. lineatus* is more frequent at sheltered ones (Williams 1965; Crothers 2001; Rubal et al. 2014). Ingrina is a very sheltered beach, and the specimens were captured in an area mostly composed of cobbles. The presence of cobbles disperses the force of waves, as the swash percolates rapidly through the sediments (Owens 1978), which means that the sampling was performed in a very sheltered area of Ingrina beach. Since *P. lineatus* is commonly found in sheltered areas the level of wave exposure in Ingrina beach was suitable for *P. lineatus* presence. However, Rubal et al. (2014) found well-established populations of *P. sauciatus* on sheltered inner shores, indicating that the abundance of *P. sauciatus* is not limited only by wave exposure. Similarly, in this study we observed *P. sauciatus* populations in sheltered areas (Porto da Baleeira, Ribeira do Cavalo and Luz). Thus, besides the exposure, other factors may influence the abundance of these species - *P. sauciatus* usually inhabits extensive, and gently sloping, rocky platforms (Ramírez et al. 2009), thus the type of substrate in Ingrina was inadequate for this southern species. This observation indicates that the type of substrate also plays a major role on the abundance of these two gastropods.

Regarding the beaches with two sampling sides (Lagosteiros North and South, and Vale dos Homens North and South), due to the dominant northwest oceanic swell (Boaventura et al. 2002), the northern side of these beaches is more sheltered than the southern one. According to literature, it would be expected to find a higher abundance of *P. lineatus* on the northern side and a higher abundance of *P. sauciatus* on the southern one. As mentioned above, there are several studies that state that *P. sauciatus* is more abundant on exposed shores and *P. lineatus* in sheltered ones (Crothers 2001). Once again, this was not observed, neither in Lagosteiros North and South nor in Vale dos Homens North and South. *Phorcus sauciatus* was more abundant in both sides of these two beaches, therefore supporting the hypothesis that the degree of exposure is not the only factor influencing these species' distribution (Rubal et al. 2014).

Prior to the sampling for this study the winter was abnormally rigorous, with strong winds and storms from December 2012 to January 2013 (IPMA, 2013). These weather conditions may have contributed to the low abundance of both species in the following summer, especially in the northern locations, since the individuals could be easily swept away by the strong currents. Boulding (1993) studied survival mechanisms of *Littorina* sp. and concluded that in the most exposed areas only juvenile snails survived the winter. This is related with their size, since they are small enough to fit in suitable refuges from extreme flows. According to Williams (1965) and Crothers (2001) *P. lineatus* migrates up-shore in summer and down-shore in winter, being this movement more evident in the larger size groups. Thus, it was expected to find at least small specimens from *P. lineatus*, but this was not observed. In February 2014, after the storm Stephanie (IPMA, 2014), there were almost none specimens of both species in Bicas and Foz, and in Avencas none young specimen of *P. sauciatus* was found, despite rigorous searching. Winter may have been too rigorous, and these species did not withstand the force of wave action. An interesting fact is that in both Bicas and Foz there were many *Gibbula* sp. specimens, which could have resisted the conditions that swiped *Phorcus* spp. in those locations.

Although there are several possible causes for the differences in *P. lineatus* and *P. sauciatus* distribution, it is important not to forget that this apparent abundance can greatly vary due to the sampling process. Additionally, taking in account that the Portuguese coast is the limit of three biogeographic regions, it is expected short-term variability in abundance of these species (Mieszkowska et al 2013).

(2) What is the influence of latitude on the variation of shell size and shape?

The shell shape did not show an evident pattern when considering all populations from the Portuguese coast. The latitudinal range of the Portuguese coast is not very wide, and throughout the coast there are several different capes, canyons and other geomorphological accidents that influence the coastal exposure (Boaventura et al. 2002), making the Portuguese coast very heterogeneous, thus a clear shape pattern is difficult to observe.

Variation was found within and between populations, and for *P. lineatus* a geographic gradient was apparent in some extent. Considering the traditional method, this species presented a tendency for broader and higher shells in the northern populations (from Papoa to Cabo Raso), to smaller sized shells in the central populations, whereas the populations from the south (from Vale dos Homens North to Luz) are grouped in a lower range of width/height. Differently, *P. sauciatus* populations presented a more uniform width, and therefore there was no evident pattern.

Results from geometric approach did not show any clear pattern throughout the coast but there were some locations clearly distinct from the others. For *P. lineatus*, Lagosteiros North was significantly different from all sites except from Lagosteiros South and Ponta Ruiva. On the other end, Porto da Baleeira was only significantly different from Cabo Raso and Lagosteiros North. The difference or similarity between these locations was in fact present, but was not visible in the distribution of the RWs (Fig.15).

As stated by de Wolf et al. (1998) macrogeographic shell variation of gastropods is not perfectly understood. Although Lagosteiros and Ponta Ruiva are far from each other, they have similar substrate, mainly composed by boulders and cobbles, which could justify why the specimens from these locations are similar between them. However there are many others locations with that same substrate, so there must be other variables that influence shell shape.

Since exposure is thought to be the primary selective force acting on shell morphology (Trussell & Etter 2001), and knowing that Porto da Baleeira is perhaps one of the most sheltered locals sampled - besides being South oriented and protected from the dominant northwest oceanic swell, the beach is positioned in a re-entrance of the mountains, and the sampling was performed on the west side of the beach, which is more sheltered than the east side - it is logical to assume that the exposure was not the main factor influencing the shell shape in this location.

In habitats with low constraints, selective pressure is low, thus allowing higher variation expression instead of a convergence towards the fittest form. In this case, the fact that Porto da Baleeira is a very sheltered area might not imply an adaptation of shell shape, and that’s why these specimens are so similar to almost all others populations. However, other south oriented locals, therefore very sheltered, like Luz and Ingrina, did not present similar results.

Therefore, in these places shell shape is probably influenced by other factors that were not taken in account, like the presence of predators or SST. These results and interpretations are very tentative and speculative, and may be confounded by microgeographic heterogeneities

For *Phorcus sauciatus*, Burrinho and Alteirinhos were the populations more significantly different from all the others. On the contrary, Avencas and Monte Clérigo were only significantly different from Burrinho, and from Burrinho and Alteirinhos, respectively.

(3) What is the influence of shore exposure on shell size and shape variation?

In this study we investigated shell morphology of two species considering shore orientation in capes, i.e., comparing all populations from the north side of all capes, with all populations from the south side of all capes, and considering population position in each cape i.e., comparing populations from the north and south of each cape. This would allow us to understand if population location translated into different shell morphology, according to the prevailing level of exposure to wave action. Note that when considering the integrated effects of shore orientation, all populations with more than 10 individuals from all capes where considered, and in the cape by cape approach only were considered the capes that had the two species, so Cape Carvoeiro was disregarded.

Typically, according to Boulding (1993), intertidal gastropods from wave-exposed shores are small and have thin shells with a large opercular aperture, whereas intertidal gastropods from protected shores are typically larger. According to Denny et al. (1985) gastropods are smaller in exposed areas since they must remain small enough to use microhabitats not exposed to the full impact of breaking waves. The increased probability of dislodgement by mechanical drag forces increases the mortality rates before the individuals reach the maximum size. Therefore the environment imposes mechanical constraints on the behaviour of the gastropods and indirectly limits their body size. Wave action also limits foraging time, because organisms cease movement to increase adhesion, since freedom of movement is limited by drag forces (Boulding, 1993; Pardo & Johnson, 2005; Brown & Quinn, 1988; Trussell, 1997), thus lowering energy available for growth and reproduction (Brown & Quinn, 1988). Like Denny et al. (1985), these authors also stated that gastropods are generally smaller at exposed sites. In their study, mean shell length of the specimens from the protected site was significantly larger than the ones from the exposed site. According to them, specimens from sheltered areas feed more than the specimens from the wave-swept areas, therefore growing more.

Contrarily to the studies mentioned above, the locations where the largest *P. lineatus* individuals were captured (Cruz dos Remédios and Portinho da Areia Norte West) are in fact exposed to the wave action. Therefore the exposure to the wave action is not limiting the size development in these populations. The common feature of these two populations is their low abundance. In the present study the large size of these individuals could be the result of low intra-specific competition due to the low density. This is in agreement with Williamson & Kendall (1981) that stated that high densities of *P. lineatus* resulted in slower growth rates (Williamson & Kendall 1981; Rubal et al. 2014), and consequently smaller individuals.

Through the traditional approach, and considering the integrated effects of shore orientation, specimens of both species are slightly broader and flatter in the north side of the capes where, due to the dominant northwest oceanic swell (Boaventura et al. 2002), the exposure is stronger comparing with the south side. Flatter shells are ideally suited to withstand water turbulence, since the increase in relative shell height decreases the gravitational stability of the shell, thus, dislodgement can occur more easily (Vermeij 1973). The populations of *P. lineatus* from the north side included larger specimens, whereas in the south specimens' size was smaller and less variable. As mentioned, in the north side of capes the abundance of the species is lower, reducing intra-specific competition for resources, thus allowing the achievement of bigger sizes (Williamson & Kendall,1981; Rubal et al. 2014). In *P. sauciatus* the sizes of the two groups are more similar, with size range overlapped.

When considering the comparisons between north and south in each cape, *P. lineatus* specimens from the north side are in general flatter than the ones from the south. In Cape Espichel however, all populations seem similar. For *P. sauciatus*, there were no differences between north and south of capes, except in Cape of São Vicente, where the specimens from the north side are clearly flatter than the ones from the south.

As mentioned before, shell shape in gastropods has been widely documented to vary according to the degree of exposure to wave action (Frid & Fordham 1994). Through the geometric approach, and considering the integrated effects of shore orientation, although the shell shape of *P. lineatus* and *P. sauciatus* is significantly different according the North-South position in the capes, this difference is small, which means shape is not markedly differentiated, hence the overlap of RW values of the southern and northern populations (Fig.16), especially in *P. sauciatus*. Regarding these results, we can infer that wave exposure on the northern and southern side of the capes is not sufficiently different to cause a differential selection and adaptation of the populations, and consequently, to result in an obvious difference of shell shape. However, in *P. lineatus* there is actually a slight difference between sides - the specimens from the north side of the capes have more variable shell shapes than in the south. In the south shell shape is less variable, and the RW distribution is more circular (Fig. 16), presenting specimens with shell shapes towards all directions of the deformation grid, without any specimens in the extremes points of deformation.

Looking to the cape by cape perspective, the shape of the two species was very variable, and a pattern was only visible in few cases. Regarding *P. lineatus*, Cape Raso was the only shore where there was a clear pattern: where the wave action is intense, the majority of the specimens had indeed a wide and high opercular aperture; and in the most sheltered beach of the cape, Avencas, the opercular apertures were narrower. In *P. sauciatus*, the shape of the specimens was even more variable, and differences were only slightly evident. In Burrinho, however, specimens had wide and high apertures, which is an unexpected shape since it is located in the south side of the Cape of Sines.

On exposed shores dislodgement by waves is considered the major cause of mortality, whereas on protected shores predation by shore crabs and desiccation are thought to be more important (Boulding 1993). Therefore, a large opercular aperture allows the development of a large foot, allowing a better fixation on the rocks, an essential feature in the species that inhabit areas with strong exposure (Wolf et al. 1997). According with Vermeij (1978) the decrease of wave action combined with increase of heat stress and predatory intensity towards the tropics influences shells to have smaller apertures. This feature is advantageous, not only to avoid predation, since it impedes the entrance of the claw of crabs, but also decreases heat stress, since contact with the substrate is reduced (Wolf et al. 1997).

The variability found in the shell shape of both species indicates that exposure may not be enough to shape these species differently. As stated by de Wolf et al. (1998) macrogeographic shell variation of gastropods is not perfectly understood.

Looking in a smaller scale, i.e., two populations from two different sides of the same beach, the specimens of *P. lineatus* from Portinho da Areia Norte West have not only a larger width range (16 mm - 25 mm), but also a more variable shape than the ones from the East side. In the latter, where width ranges from 14 to 18 mm, shell shape is less variable, and there are specimens with small opercular aperture.

Althougth located in opposite sides, Portinho da Areia Norte East and West do not have much different exposure to the wave action. The predominant oceanic swell comes from the northwest, so both places are exposed similarly to the wave action, therefore supporting the hypothesis that the degree of exposure is not the only factor influencing these species' distribution.

In Lagosteiros North, where the smaller individuals were captured, the shell shape was more variable, with specimens with high and narrow apertures. In both Lagosteiros North and South there were no individuals with wide aperture. It was also the North side of Vale dos Homens North that presented specimens with more variable shape, and there were no specimens with short aperture. In the south side of this beach the shape was less variable, and concentric with the centre of the RWs axis (Fig. 17).

Regarding *P. sauciatus* there were no differences of width and height between the two sampled sides. However, through the geometric approach, there were differences between the sides. In Vale dos Homens North shell shape was more variable, and the opercular aperture was less wide than *P. lineatus*. Actually the apertures were high and narrow. In the south side the shell shape was less variable, almost in the centre of the distribution (Fig. 18), and specimens had wider apertures than in the north side.

Curiously in the same beach, Vale dos Homens, the two species are very different. In the case of *P. lineatus* we observed a strong linear relation between shell width and height, whereas for *P. sauciatus* this relation was not present, and an increment of shell width was not accompanied by an increment on shell height.

(4) Does shell shape vary during ontogeny?

In both species, as individuals grow, the relations between width and height become more disperse. In *P. lineatus* for the first two size classes the relation is almost linear; and in the last two classes, even more evident in the fourth, the specimens are disperse, i.e. the width varies more independently from the height. In the other species the relation between width and height is always more disperse.

As organisms grow, they adjust and adapt themselves to the environment, resulting in a variation of the shell shape - as the shell grows the volume of the shell increases more rapidly than the area of the shell opening. Therefore, in order to maintain a favourable ratio between area of the aperture and volume of the shell, it is expected for the gastropod to alter its geometry during ontogeny (Raup 1966).

In *P. lineatus*, both in Class I [1 mm - 5 mm] and IV [16 mm - 20 mm] there are no individuals with wide apertures, and, on the other hand, both Class II [6 mm - 10 mm] and III [11 mm - 15 mm] do have wide apertures. The youngest and the old specimens are similarly exposed to the wave action: both classes were found in small pools in the upper zone of Avencas, therefore protected from the wave action. Phenotypic plasticity, which is the capacity of an organism to produce different phenotypes in response to environmental cues, can be an important adaptive strategy in variable environments, because different conditions can lead to different adaptations, and consequently to different phenotypes. Since the risk of dislodgement is low, there is no need in developing a large foot, and, consequently, the aperture is also small. To be adaptive, the plasticity of a feature must confer benefit to the organism, such as improved survival, growth, or reproduction. Therefore, the plastic increase in foot size may be adaptive in reducing the risk of dislodgment, because a larger foot promotes increased adhesive ability, and consequently improves survival in a hydrodynamic stressful environment (Trussell 1997).

As mentioned before, in habitats with low constraints, selective pressure is low, thus allowing higher variation expression instead of a convergence towards the fittest form, i.e. phenotypic plasticity is not always adaptive, and may simply reflect the non-adaptive impact of the environment on the physicochemical processes of the organism

Regarding *P. sauciatus*, individuals from Class II are very flat, with large and wide apertures; from the Class IV the apertures are smaller; and concerning Class III, it is almost in the centre of the graph (Fig 20).

FINAL REMARKS

This study contributes and complements the information available in the scientific community in relation to morphological variation of *Phorcus lineatus* and *Phorcus sauciatus*, which may help to better understand the mechanisms of variation in other gastropods.

The morphology of the shell of these two species of intertidal gastropods was analysed in order to uncover how does the shell varies during the ontogeny, and how latitude and wave exposure may influence shell shape and size. A traditional and a geometric approach were used. There was no clear pattern in the results for all of these questions.

Shell morphology is clearly influenced by several variables, and some were not taken into account in the present study, thus, it would be interesting in future studies to evaluate shell morphology taking into account other variables such as the type of substrate, presence of other species (predators or competitors), or the quantity of available food. Also, a design involving the assessment of variability at different spatial scales would be important to clarify spatial heterogeneities.

Another aspect important to refer is that the latitudinal range of the Portuguese coast is not very wide, thus, probably not providing a gradient of environmental conditions (such as temperature or wave action) enough to produce a remarkable shell differentiation along the coast. It would be interesting to analyse specimens collected in the limits of their distribution. Lastly, combining data from genetic and morphological approaches would be interesting since genetic structure might be reflected in phenotypic differences.

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